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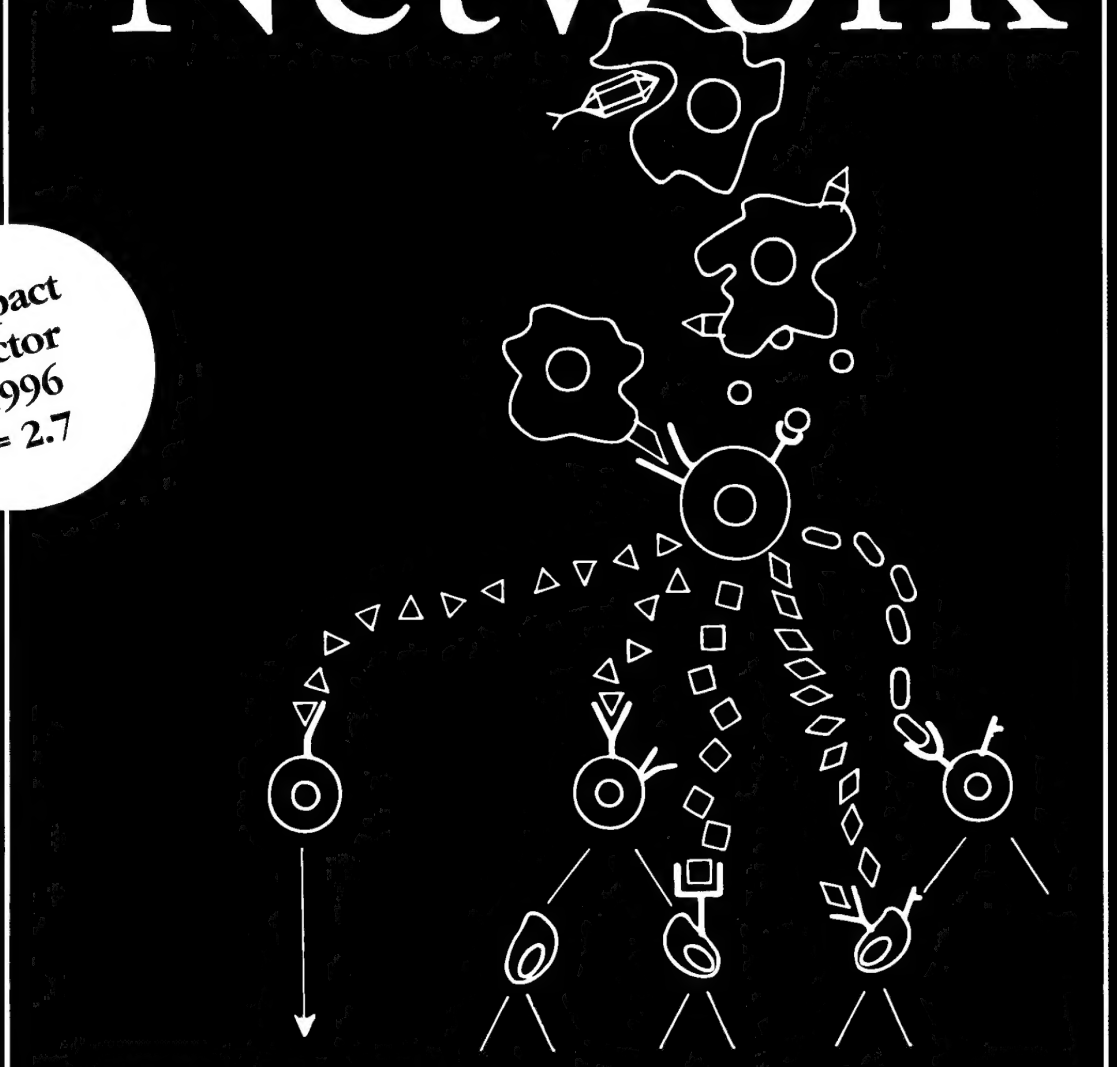

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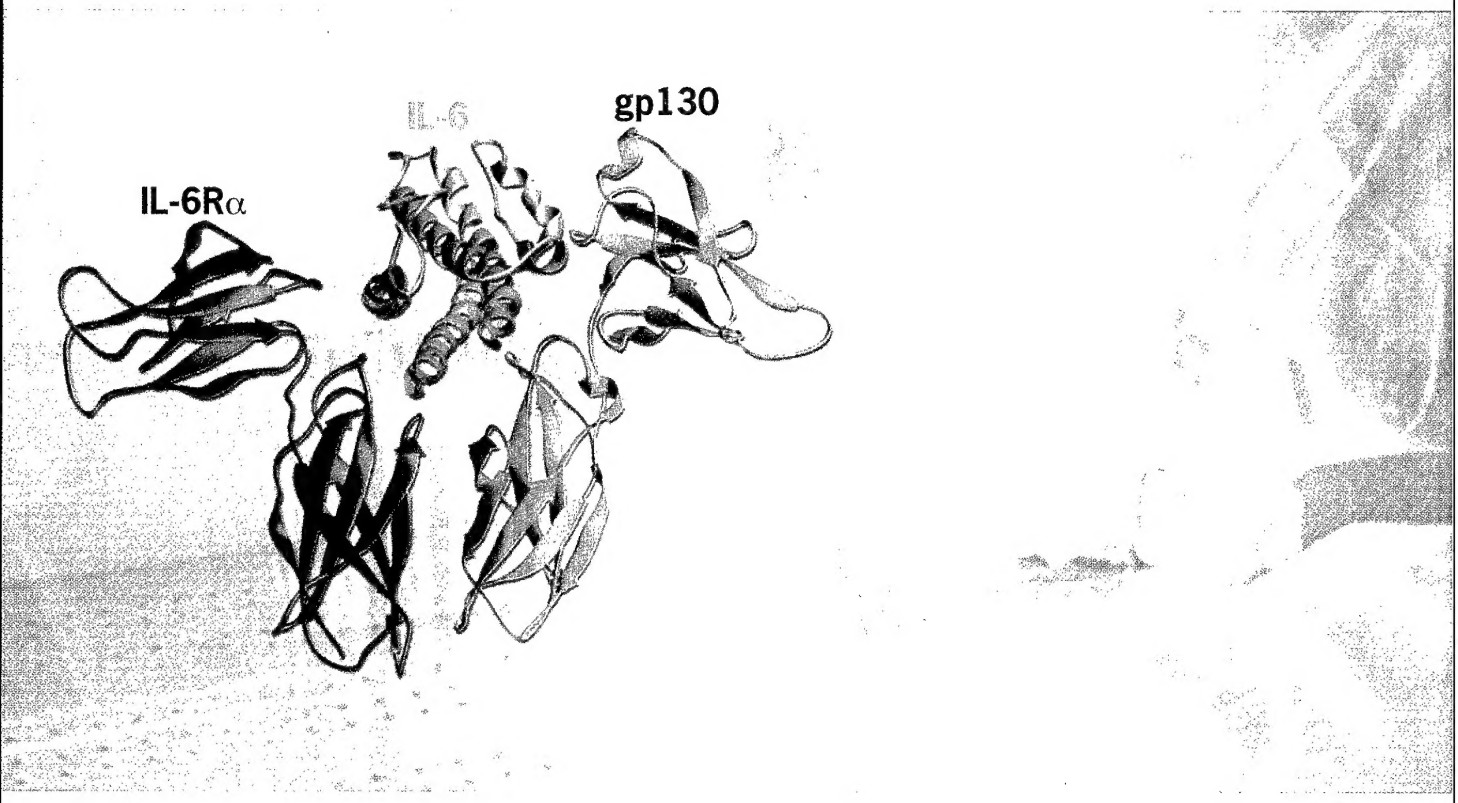

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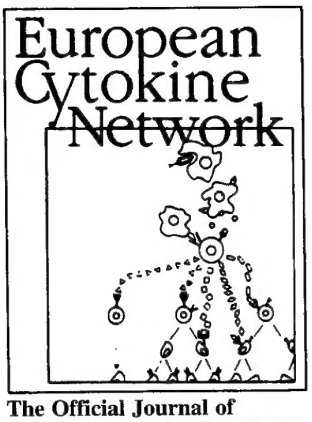

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THIRD JOINT MEETING OF THE ICS AND THE ISICR

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Summary of Symposia at the Twenty-Third Annual Conference on Shock held in Snowbird, Utah June 3-6, 2000
Summary of Symposia at the 8th International Cytokine Conference held in Amsterdam, November 5-9, 2000.
Both Symposia were very successful. The attached Summaries describe the state-of-the-Art Scientific progress being made in these vital areas. Also attached are abstracts of all the papers delivered at the Conference.

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February 19, 2001

Dr. Jeannine Majde
Program Officer, ONR
Office of Naval Research
Ballston Tower One, Code 335
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Arlington, Virginia 22217-5660

RE: Grant N00014-00-1-0708

Dear Dr. Majde,

On behalf of the Scientific Program Committee, Officers and Council of the Society, I want to thank the Office of Naval Research for the Support of Symposia and Awards at the Twenty-Third Annual Conference on Shock, June 3-9, 2000, Snowbird, Utah and the Eighth International Cytokine Conference, November 5-9, 2000, RAI, Amsterdam.

These meetings were very successful and attended by scientists and physicians throughout the world. We are grateful for the support of the Department of the Navy which helped make this meeting possible.

I am enclosing a copy of *SHOCK* Volume 13, 2000 supplement which contains the program and abstracts (pages 1- 68) for the Shock Conference. A summary of the meeting is also enclosed.

I am enclosing a copy of *EUROPEAN CYTOKINE NETWORK* Volume 11, November, 2000, which contains the abstracts (pages 8-236) for the Cytokine Conference. A summary of the conference is also enclosed.

I look forward to a continued association of the Society with the Office of Naval Research.

Sincerely,

Sherwood M. Reichard
Executive Director

Enclosure

AWARDS
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ICS Honorary Life Membership
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Postdoctoral Investigator Award: 2nd prize
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Outstanding Scholar Award: 2nd prize
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The ISICR extends its sincere recognition to the Milstein Family Foundation for support of the Milstein Award and the Young Investigator Awards.

Christian Fleischmann Memorial Award for Young Women Investigators

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The following four applicants have been selected as winners of an ECS student travel award

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Topic Index

Reviews

01005

Modulation of Chromatin Structure Regulates Cytokine Gene Expression during T Cell Differentiation

Suneet Agarwal, Orly Avni, and Anjana Rao, Department of Pathology
Harvard Medical School and The Center for Blood Research

Differentiating cells undergo programmed alterations in their patterns of gene expression, which are often regulated by structural changes in chromatin. We have shown that T cell differentiation results in long-range changes in the chromatin structure of effector cytokine genes, which persist in resting Th1 and Th2 cells in the absence of further stimulation. Differentiation of naive T helper cells into mature Th2 cells is associated with chromatin remodelling of the IL-4 and IL-13 genes, whereas differentiation into Th1 cells evokes remodelling of the IFN-γ but not IL-4 or IL-13 genes. IL-4 locus remodelling is accompanied by demethylation and requires both antigen stimulation and STAT6 activation. Chromatin remodelling of cytokine gene loci is functionally associated with productive T cell differentiation, and may explain the coordinate regulation of Th2 cytokine genes.
By DNase I hypersensitivity analysis, we identified an inducible, cyclosporin A-sensitive enhancer located 3' of the interleukin-4 (IL-4) gene. The enhancer binds the Th2-specific transcription factor GATA3 in vivo, but is not perceptibly influenced by the absence of a second Th2-specific factor, cMaf. The antigen-inducible transcription NFAT1 factor binds the IL-4 enhancer and the IL-4 promoter only in stimulated Th2 cells; conversely, NFAT1 binds to the interferon (IFN)-γ promoter only in stimulated Th1 cells. Our results support a model whereby transcription factors such as NFAT1, which are nonselectively induced in antigen-stimulated T cells, gain access to cytokine regulatory regions only in the appropriate subset of differentiated T cells in vivo. This restricted access enables antigen-dependent and subset-specific transcription of cytokine genes.

01025

IL-18

Charles A. Dinarello M.D., Division of Infectious Diseases, University of Colorado, School of Medicine, Denver, Colorado, USA

Abstract not available at time of printing.

10049

Josef Penninger, Ontario Cancer Institute, University of Toronto, Toronto/Ontario, USA

MAMMALIAN EVOLUTION, BONE AND T CELLS

10050

UNDERSTANDING MULTIPLE SCLEROSIS: THE OUTLOOK FOR NOVEL THERAPEUTICS BASED ON CURRENT KNOWLEDGE.

Richard M. Ransohoff, M.D.
The Mellen Center for Multiple Sclerosis Treatment and Research
Dept. of Neurosciences, The Lerner Research Institute
Cleveland Clinic Foundation, Cleveland, OH

The past decade has seen dramatic, historic changes in understanding the pathogenesis and treatment of multiple sclerosis (MS). At present, MS is viewed as a destructive process of the human central nervous system (CNS), initiated by inflammatory demyelination but including prominent axonal pathology, revealed both by advanced imaging techniques and traditional histopathology. Mechanisms of myelin destruction have been clarified and proposed therapeutics for MS now include neuroprotectants.
Serial gadolinium-enhanced magnetic resonance (MR) scans revealed MS as a continuously-active process. Brain and spinal cord atrophy, defined by MR, were shown to correlate closely with clinical state. MRI techniques therefore are now considered "gold standard" tools for monitoring disease activity and severity. A task force of the National Multiple Sclerosis Society developed improved complementary methodology for clinical assessment during therapeutic trials.
These efforts have already produced improved therapy for MS patients: two categories of agent, interferon-beta and glatiramer acetate, were shown to slow radiographic and clinical progression. What's next? It is critical to determine how axonal pathology is produced in MS lesions. We need longitudinal cost/benefit analysis of current treatments (which are expensive and parenteral). Biochemical, immunologic and electrophysiologic monitoring techniques are eagerly awaited. New treatments under active development include small-molecule antagonists of chemokine receptors. Our knowledge of the functions of these receptors in MS is embryonic but growing rapidly. Given the current pace, we may hope that the first ten years of the 21st century will be as productive as the last ten years of the twentieth.

19003

F. Melchers, Basel Institute for Immunology, Basel, Switzerland

Abstract not available at time of printing.

19008

ROLE OF INTERFERON-INDUCED PROTEINS IN INNATE IMMUNITY

Bryan R. G. Williams, Kee Chuan Go, Michael de Veer, Aristibolo Silva, Michelle Holko, Mathias Frevel, and Robert H. Silverman. Department of Cancer Biology, Lerner Research Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue Cleveland Ohio 44195

Interferons (IFN) regulate the expression of several hundred genes that can be divided into different functional groups. The groups most represented play important roles in innate immunity and including select transcription factors, signal transduction molecules, components of the proteosome, and cell surface receptors. These interferon stimulated genes (ISGs) have been assembled into a searchable database and arrayed as cDNAs for use in a range of experiments that measure IFN action. Many genes have yet to have a specific function ascribed to them but cluster analysis of transcription profiles can sometimes provide clues to activities. Studies with knockout animals can reveal heretofore-unrecognized functions of proteins that may have been thoroughly studied only at a biochemical level. Arguably, the subject of most recent attention is the double-stranded RNA-dependent kinase PKR. Studies of the phenotype of this knockout revealed selective functions for PKR in signaling via different pathways including not only dsRNA but also TNF-alpha, IL-1 and LPS. The contribution of PKR in these signaling pathways varies and may or may not require its enzyme activity. While the direct targets of PKR remain to be defined, p38 MAP kinase, NF-kappaB, and ATF2 activity are diminished in the absence of PKR and the induction of proinflammatory cytokines decreased. The link between TNFR or Toll-like receptors to PKR remains to be determined as does the contribution of PKR to disease induction by pro-inflammatory agents. In the case of virus infection, PKR along with RNaseL contribute to much of the protection conferred by IFN to highly cytolytic viruses. In addition PKR is also responsible for determining resistance to tissue restricted infection in the lung probably through regulating induction of nitric oxide synthase. The induction of E-selectin by proinflammatory stimuli is also regulated by a PKR-dependent pathway indicating another point of intersection between this IFN-regulated protein and innate immunity may be reflected in cellular trafficking.

28007

EVOLUTION OF CYTOKINES:
Identification and possible function in the phylogenetically oldest metazoans the sponges

Werner E.G. Müller
Institut für Physiologische Chemie, Abteilung Angewandte Molekularbiologie, Universität, Duesbergweg 6, D-55099 Mainz

Porifera (sponge) form the lowest metazoan phylum and share a common ancestor with other metazoan phyla. Recently we reported that sponges possess molecules that are similar in structure to those molecules involved in the immune system in mammals. Experiments with the marine sponges *Geodia cydonium* and *Suberites domuncula* have been performed on tissue as well as on a cellular level. The studies revealed that sponges are provided with elements of the mammalian innate immune system. Furthermore, macrophage-derived cytokine-like molecules have been identified which are upregulated during the grafting process. The allograft inflammatory factor 1 (AIF-1) was identified in *S. domuncula*. The expression of this sponge gene as well as of the sponge glutathione peroxidase (GPX) was found to be strongly upregulated in allografts after a 2 days incubation period. This finding indicates that the strong expression of the AIF-1-like protein and GPX occurs in allografts, suggesting a possible function in activating "immunocytes" to alloimmune rejection. Other potential sponge cytokine-like molecules have been identified and cloned in sponges; e.g. endothelial-monocyte-activating polypeptide type II. Until recently, (2-5)A synthetases have been identified enzymatically or by molecular cloning only in vertebrates. In non-vertebrate systems the product of the (2-5)A synthetase has been unequivocally characterized in the sponge *G. cydonium* both by biochemical and immunological methods. Recently, the (2-5)A synthetase gene was cloned from this sponge. It is concluded that sponges are already provided with cytokines active in innate and adaptive immune recognition.

28076

TOLL-LIKE RECEPTOR 4 AND MD-2 FUNCTION AS A RECEPTOR COMPLEX TO ENABLE LPS RESPONSES AND IMPART LIGAND-SPECIFIC RECOGNITION OF BACTERIAL LIPOPOLYSACCHARIDE

Douglas T. Golenbock, Egil Lien*, Andra Schromm, Atsutoshi Yoshimura#, Holger Heine**, Terry Means, Matthew Fenton, Shoichi Kusumoto¥ and Kensuke Miyake+. Boston University School of Medicine, Boston, USA; * Norwegian University of Science and Technology; #Nagasaki Univ. School of Dentistry, Japan; **Research Center Borstel, Germany; ¥Osaka University, Japan; +Saga Medical School, Japan.

Recent evidence suggests that LPS-induced signal transduction begins with CD14-mediated activation of one or more Toll-like receptors (TLRs). Expression of Toll-like Receptor (TLR) 4 appears to be an essential feature of all cell types that can be activated by enteric preparations of purified endotoxins. By combining molecular genetic approaches with pharmacological studies of lipid A-like molecules, we have observed that TLR4 expression is sufficient to enable CD14-enhanced LPS responses and define the specificity of ligand recognition. In addition, studies of LPS non-responder mutant cell lines, isolated from highly responsive Chinese hamster ovary (CHO)-K1 cells that express human CD14 (CHO/CD14), suggest that expression of the secreted protein MD-2 is also an essential component of the LPS receptor system. CHO/CD14 cells that carried a single base-pair missense mutation for MD-2 were refractory to LPS stimulation unless complemented by gene transfection techniques with the MD-2 cDNA. Our data support a model of the LPS receptor that consists of CD14, TLR4, and MD-2. Analysis of a second complementation group of CHO/CD14 mutants suggests that the LPS receptor appears to consist of at least one additional molecule.

28077

INTERFERONS AND CANCER 2000: WHERE FROM HERE?

Ernest C. Borden, Cleveland Clinic Foundation, Cleveland Ohio 44195 USA

Interferons (IFNs) are the most broadly effective cytokine for treatment of neoplastic disease. To reach their full clinical potential, the following problems need to be addressed. 1) Definition, correction, and circumvention of resistance mechanisms in signal transduction pathways. Stat 1 activation and expression is only one component. 2) Clinical and preclinical leads with IFNs other than IFN-α2, the only widely approved IFN for malignant disease, need to be more aggressively clinically evaluated. Molecules, such as IFN-α1, may be better tolerated. IFN-β, which induces apoptosis more effectively *in vitro* in melanoma than IFN-α2, has yet to be tested as a single agent at its clinical MTD. Pegylated IFNs clearly have an altered pharmacologic profile. 3) Identification of antitumor mechanism(s) of action. Apoptosis *in vitro* in melanoma is likely resulting from TRAIL. But gene arrays continue to define new interferon-stimulated genes, some of which seem histology specific. 4) Integration with other therapies. Opportunities will expand for use in combination with signal transduction modulators in CML and lymphomas. Data continue to suggest advantage of IFN-α2 in combination with surgery for early melanomas and bladder carcinomas and advanced renal cell carcinoma. Once research initiatives provide answers to these preclinical and clinical questions in the next decade, further decreases in cancer morbidity and mortality will result.

Cytokines and T-cell differentiation

01008

Exposure of maturing DC to IFN-γ results in their stable Type-1-polarized effector phenotype

Pedro L. Vieira,[1] Esther C. de Jong,[1,2] Eddy A. Wierenga,[1] Martien L. Kapsenberg[1,2] and Pawel Kalinski[1,]*[1]Dept. of Cell Biology and Histology and Dept. of Dermatology, Academic Medical Center, University of Amsterdam, PO Box 22700, 1100 DE Amsterdam, The Netherlands.*

IL-12 is a key APC-derived cytokine that promotes Th1 responses. The levels of IL-12 produced by mature DC during the interaction with naive Th cells (ThN) affect the development of polarized Th1 and Th2 subsets. Several factors, such as IL-10, PGE_2, and glucocorticoids, are known to induce IL-12-deficient DC that promote Th2 responses. In contrast, the identity of factors that can induce a stable Th1-promoting effector DC is unknown. Here we show that IFN-γ, a product of activated NK cells, can induce such Type-1-polarized DC. The presence of IFN-γ during LPS- or IL-1β/TNF-α-induced maturation of human monocyte-derived DC did not affect the appearence of CD83 and the high expression of CD40, CD80, CD86 and HLA-DR. However, IFN-γ profoundly increased (by 2 orders of magnitude) the ability of maturing DC to produce IL-12 upon subsequent CD40 ligation. Furthermore, while the induction of detectable IL-12 production in control DC by ThN required the additional presence of exogenous IFN-γ, the interaction of ThN with IFN-γ-pre-exposed DC resulted in substantial IL-12 production independently of any additions. The high-level IL-12-producing capacity of IFN-γ-exposed DC, acquired during their maturation, is resistant to further modulation, resulting in a stable Type-1-effector DC with a potent Th1-inducing capacity. In contrast, DC matured in the presence of PGE_2 display a Type-2 effector phenotype, inducing the development of Th2 cells. It is concluded that human myeloid DC maturing in different environments can carry an additional message to the lymphoid tissues, determining the class of the immune response. Effector Type-1-polarized DC may be candidates for Ag-specific induction of therapeutic Th1 responses in cancer and in chronic infections with intracellular pathogens.

01003

Pathogens evoke protective Th1/Th2 responses via the induction of type 1 and type 2 effector DC

E. C. de Jong[1,2], P. Kalinski[1], J.H.N. Schuitemaker[1], P.L. Vieira[1], E.A. Wierenga[1], M. Yazdanbakhsh[3] and M.L. Kapsenberg[1,2]. [1]Dept of Cell Biology & Histology, [2]Dept of Dermatology, AMC, UvA, Amsterdam, [3]Dept of Parasitology, LUMC, Leiden, The Netherlands

Efficient elimination of pathogens requires specific Th1 or Th2 responses depending on the type of invading pathogen. The way pathogens evoke the protective Th phenotype is not fully understood. Dendritic cells (DC) are key initiators of primary T cell responses. Previously, we have demonstrated that pathogen-induced tissue factors, present at the time of activation of uncommited immature DC induce the maturation of effector DC with Th1 cell promoting capacities (type 1 DC or DC1) or DC with Th2 cell promoting capacities (type 2 DC or DC2). In this respect, IFN-γ induces the development of DC1, which produce high levels of IL-12 and express high levels of ICAM-1. The presence of PGE2 leads to IL-12-deficient DC2 which express OX40L. However, the question remained whether pathogens can also induce the development of polarized effector DC in a direct fashion. Therefore, the polarization of DC into DC1 or DC2 by pathogens from different origens was studied. Indeed, a bacterial toxin derived from the extracellular bacteria *Vibrio cholerae,* induced the development of IL-12-deficient DC2, which will induce a protective Th2 cell respons. A toxin derived from the intracellular bacteria *Bordetella pertussis* induced the development of DC1 with an enhanced capacity to produce IL-12 production which will promote a protective Th1 cell respons. Poly I:C (dsRNA, a mimick for virus) induced the development of extremely potent Th1-inducing DC1 without, however, an enhanced capacity to produce IL-12. An extract derived from the eggs of the helminth *Schistosoma mansoni* induced the development of strongly polarized effector DC2, in occordance with the requirement of an antibody-dependent (Th2) response for efficiently elimination of the infection. This study demonstrates that pathogens not only indirectly polarize the DC phenotype via induction of tissue factors but also via direct activation of sentinel DC into effector DC1 or DC2.

01002

PANCREATIC CELL CARCINOMA-DERIVED INTERLEUKIN-10 AND VASCULAR ENDOTHELIAL FACTOR DIFFERENTIALLY AFFECT DENDRITIC CELL DEVELOPMENT.

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Dendritic cells (DC) are professional antigen-presenting cells (APC) required for the initiation of primary T-cell response in vivo. It has been suggested that tumor cells produce a variety of immunomodulatory cytokines affecting the host response to tumor cells. The escape of malignant cells from the immune response against the tumor may result from a defective development or function of DC. The present study was performed to explore the effects of two of such cytokines, aberrantly produced by pancreatic tumor cells, Vascular Endothelial Growth Factor (VEGF) and Interleukin (IL)-10, on the differentiation of the two distinct types of DC precursors, pDC1 and pDC2. Exogenous VEGF (100 ng/ml) was found to inhibit preferentially the differentiation of DC2 derived from $CD4^+CD3^-CD11c^-$ cells cultured with IL-3 for 6 days. By contrast, IL-10 (20 ng/ml) prevents the generation of DC1 from monocytes cultured for 7 days with granulocyte-macrophage colony stimulating factor (GM-CSF) and Interleukin (IL)-4: Similar results were obtained using conditioned media of three different pancreatic carcinoma cell lines. Pretreatment of supernatants with neutralizing antibodies against IL-10 and VEGF restores the DC1 and DC2 differentiation. Since "lymphoid" DC2 induced Th2 differentiation, whereas monocytes-derived DC1 were found to induce Th1 differentiation, we can speculate that the tumor, by the combined release of VEGF and IL-10, may hamper the development of the two distinct subsets of CD4+ cells, both *required in generating an effective anti-tumor immune response.*

01006

Transcription factors T-bet and GATA-3 are reciprocally involved in human Th1 and Th2 cell polarisation.

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Th cells can differentiate along two distinct pathways, yielding Th1 or Th2 cells, as defined by distinct cytokine profiles and functional properties. It is well established now that the cytokines IL-12 and IL-4 play a key role in polarization of naive T cells into Th1 and Th2 cells, respectively. At this moment, much attention is drawn to the molecular basis for the subset-restricted expression of cytokine genes. In the mouse, several Th2-specific transcription factors have been described, including GATA-3, whilst more recently, a Th1-specific transcription factor, T-bet, was identified. The aim of our study was to analyze the expression levels of GATA-3 and T-bet in human Th1 and Th2 cells and to establish their function in T cell polarization. To that end we analyzed GATA-3 and T-bet mRNA levels by real-time PCR in polyclonal Th1 and Th2 cell lines, as well as in *M.Leprae*-specific Th1 cell clones and housedust mite-specific Th2 cell clones, all 24h after stimulation. Similar to mouse cells, high levels of GATA-3 but only trace amounts of T-bet were demonstrated in all Th2 cells tested. In contrast, Th1 cells hardly expressed GATA-3, but expressed T-bet abundantly. We next analysed the regulatory influence of the polarizing cytokines IL-4 and IL-12 on the expression of these transcription factors in naive T cells. During stimulation of naive T cells in the presence of exogenous IL-4, GATA-3 was strongly upregulated both on mRNA and protein level. In contrast, IL-4 did not effect T-bet expression. Stimulation in the presence of IL-12 gave rise to high expression levels of T-bet and in addition strongly suppressed GATA-3 levels in comparison to neutrally stimulated naive T cells. Furthermore, restimulation of effector Th2 cells in the presence of IL-12 was accompanied by the complete dissappearance of GATA-3 protein, both in the cytoplasm and in the nucleus, whilst T-bet was abundantly upregulated. This study suggests the involvement of the transcription factors GATA-3 and T-bet in the polarization of human Th1 and Th2 cells and provides preliminary information on the regulation of the expression of these factors.

01004

Modulation of IFN-α/β sensitivity upon IL-12 receptor up-regulation in human T cells

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In humans, type I interferons (IFN-α/β), in addition to IL-12, have been shown to activate STAT4, to upregulate the β2 chain of the IL-12 receptor and to play an important role in driving the differentiation of Th1 cells. In this study, we have compared the IFN-α and the IFN-β sensitivity of naïve precursors and *in vitro* differentiated Th1/Th2 cells obtained from human cord blood leukocytes. The steady-state mRNA levels of two IFN-α/β inducible genes (6-16 and MxA) were measured by quantitative real-time PCR. While naïve T cells were highly responsive to picomolar concentration of IFN-α and β, a significant reduction in the IFN response was observed in both Th1 and Th2 cells.

Up-regulation of the IL12 receptor β1 and β2 chains occurs upon activation of naïve T cells. Notably, the β1 chain, whose surface expression is maintained on both differentiated Th1 and Th2 subsets, interacts with the tyrosine kinase Tyk2, which has been previously shown to also associate and sustain the level of the IFN-α/β receptor chain IFNAR1. Thus, up-regulation of the β1 chain in Th1/Th2 cells could sequester Tyk2 and be partly responsible for their reduced IFN sensitivity. Results obtained in reconstituted fibroblastic cells expressing exogenous β1 and/or β2 support this hypothesis and suggest that trans-modulation of cytokine signaling could occur through competition of receptors for a common Jak protein.

01007

Differential response of CD4+ and CD8+ T cells to IL-12 or IL-18 depending on the immune status in tumor-bearing patients

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The IL-12 and IL-18 responses were investigated in tumor bearing patients and healthy individuals through IFN-γ production. CD56+ (NK / NKT) cell-enriched population responded similarly to IL-12 and IL-18 both in cancer patients and healthy controls. In contrast, in IL-12 response of $CD4^+$/ 8^+ T cells, there was a remarkable difference between tumor -bearing patients and controls. T cells from healthy individuals produced negligible or small amounts of IFN-γ in response to IL-12. Conversely, considerable portions of $CD4^+$/8^+ T cell populations from tumor -bearing patients exhibited moderate to high IL-12 responsiveness. Most of IL-12 responding T cells were also found to respond to IL-18. In contrast, in healthy controls, almost null IL-18 responses were observed. Expression of CD25 in T cells from tumor-bearing patients was significantly higher than those from healthy individuals. Even in non IL-12 responsive T cells from either cancer patients or healthy subjects, if stimulated with anti-CD3 plus anti-CD28, remarkable responses to IL-12 and IL-18 were induced. It can be concluded that IL-12 responsiveness is inducible in T cell receptor (TCR)-triggered T cells.

These results indicate that the high incidence of T cell populations from tumor-bearing patients, which exhibit IL-12 / IL-18 responsiveness, could reflect the existence of TCR-sensitized T cells. Additionally, it should be noted high responders to direct IL-12 stimulation were detected in large tumor bearers and null to low responders were shown in small tumor bearers or late stage cancer patients. IL-12 response may represent different stages in the development of tumor-associated immunity.

01001

MODULATION OF Th2 TOWARDS Th1 CYTOKINE PROFILES BY FLAVONOIDS IN PATIENTS WITH ALLERGIC ASTHMA

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Modern strategies in the therapy for allergic inflammatory diseases have focused on down-regulation of Th2 concomitantly with up-regulation of Th1 cytokine profiles. New consideration is being given to essential nutrients such as flavonoids for their protective effects against oxidant-mediated inflammation and tissue damage. Based on these data, this study assessed the capacity of some flavonoids to mediate the conversion of $CD4^+$ T helper cells inappropriately secreting Th2 cytokines towards a moderate Th1 immune response in allergic asthmatic patients. Therefore, using ELISA kits, the modulatory effects of apigenin, quercetin and rutin on Th1/Th2 cytokine production following mitogenic activation of peripheral blood mononuclear cells (PBMC) of allergic patients and healthy donors were analysed. Our data showed that apigenin treatment of PHA-stimulated PBMC of allergic patients down-regulated the increased levels of IL-4 and IL-10 while IFN-γ and IL-12 levels were enhanced. Showing a moderate inhibitory effect on Th2 cytokine profiles, quercetin markedly up-regulated IL-12 production, which could direct towards a Th1 immune response. In PHA-driven PBMC cultures from healthy donors developing an effective Th1 response, apigenin and rutin revealed an opposite effect, moderately decreasing IFN-γ whilst augmenting IL-4 production. In addition, the scavenging activity of flavonoids on reactive oxygen species released by aggregated IgG-activated neutrophils isolated from normal and allergic asthmatic subjects was analysed by chemiluminescence assay. All these data suggested that nutritional anti-oxidants such as flavonoids might be therapeutically beneficial as supplement in the treatment of allergic inflammatory diseases by reversing the enhanced Th2 response to a protective Th1 immune response.

01010

ANALYSIS OF CYTOKINE GENE EXPRESSION IN CELLS PRODUCING FACTOR THAT INDUCES MIGRATION OF STEM ELEMENTS FROM MARROW TO THE PERIPHERY.

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Previously we have shown that $CD4^-8^-$ (DN) cortison-resistant thymocytes produce chemotactic factor, inducing migration of stem elements from marrow to different lymphoid organs.

In this study we have examined the expression of a panel of cytokine genes in these cells. The cytokine response was evaluated by the appearance of corresponding mRNA transcripts as detected by reverse transcription of total RNA followed by PCR amplification of cDNA fragments using oligonucleotide primers (RT/PCR). We used oligonucleotide primer pair specific for interleukines (IL) –2,3,4,7,10, TNFα, INFγ, TGFβ, GM-CSF, SCF.

It was shown that transcripts of IL-2,3,4,7,10 and GM-CSF as well as SCF were undetectable. Weak bands of PCR–products corresponding to the sequences of INFγ, TGFβ and TNFα were detectable.

The pattern of cytokine mRNA transcription in DN thymocytes suggest that these cells do not produce cytokines with colony-stimulating activity (SCF, IL-3, GM-CSF) or capability to regulate migration of marrow cells (IL-2,4,7). Thus, these data have shown that DN cortison-resistant thymocytes produce chemotactic factor, different from analysing cytokines.

01011

FUNCTIONAL DEFECT OF THE TH2 SUPOPULATION IN CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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In chronic lymphocytic leukemia of the B-cell type (B-CLL) a reduction of T-cell numbers is commonly seen during disease progression. This phenomenon is even enhanced if patients are receiving chemotherapy (i.e. Fludarabine). As a consequence, not only tumor cells are reduced, but also CD4-positive T-cells and immunodeficiency is commonly seen in those patients. To investigate whether a particular T-helper cell subpopulation is preferentially affected in B-CLL, we studied *in vitro* T cell functions in B-CLL. In a first set of experiments antigen and mitogen-induced proliferation was analyzed. Results revealed a marked reduction of proliferation in MNC's and CD4-positive T cells derived from B-CLL. To study the capacity of T helper cell function for PWM-induced B cell differentiation, cells were cultured for 6 days and secreted immunoglobulins were measured by ELISA. Results of these experiments revealed a diminished T-helper cell activity in B-CLL. However, if autologous CD4-cells are added back, Ig-synthesis is restored, indicating that at least in this type of assay reduced T-helper cell capacity is a quantitative rather than a qualitative problem. In order to differentiate between TH1 and TH2 functions in B-CLL, MNC's and CD4 cells were stimulated with mitogens (PHA, PWM) or PMA and calciumionophore (CaIp) and cytokines specific for either TH1 (IFN-γ) or TH2 (IL-4) were assayed by ELISA. While IFN-γ was produced by both MNC and CD4-positive T cells, IL-4 was not detectable. To rule out the possibility, that secretion is inhibited, intracellular staining of these cytokines was performed. Again, IFN-γ was detectable by FACS-staining regardless of the stimulus. However, IL-4 was detectable in only a low percentage of cells (<3%) after PMA+CaIp treatment. These results show that B-CLL is associated with a diminished reactivity of T-cell functions and a defective TH2-specific IL-4 production.

01012

THE EFFECT OF ACTIVATION OF PROTEIN KINASE C ON CYTOKINE PRODUCING CELLS

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It has been established that production of IL2 and gamma interferon (IFN) is greatly augmented by PKC activation while that for IL4 is not. We questioned if these enhanced yields are accompanied by increased numbers of cytokine producing cells or increased output of cytokines by the same cells. Using FACS we have shown that the number of cytokine producing cells does increase for IL2, IFN and IL4 when PMA (a PKC activator) is added to A23187. This is true for CD_4, CD_8 and CD_{56}. Furthermore, the output of cytokines by individual cells also appears to increase using mean fluorescent intensity (MFI) of the cells as an indicator. Surprisingly however, the temporal pattern of the increase in the number of cytokine producing cells differs between CD_4, CD_8 and CD_{56} cells but not between cytokines. However, the temporal pattern of the increase in MFI differs between cytokines and not cell types. Thus, the effect of PMA on MFI is much greater on IFN than IL2 and IL4 despite the fact that IL2 and IFN yields are both subtantially affected by the presence of PMA. Of additional significance is the fact that the temporal changes in both percentage of cytokine secreting cells and MFI is substantially different when PHA alone is used to induce the cells as opposed to A23187 plus PMA. We believe these differences may suggest ways of pharmacologically manipulating specific cytokine yields or selectively stimulating or inhibiting certain cell types.

01021

IL-1 enhances T cell-dependent antibody production and T cell-priming through induction of CD40L and OX40 on T cells

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IL-1 is a proinflammatory cytokine that plays pleiotropic roles in host defense mechanisms. We investigated the role of IL-1 in the humoral immune response using gene-targeted mice, IL-1$\alpha^{-/-}$, IL-1$\beta^{-/-}$, IL-1$\alpha/\beta^{-/-}$ and IL-1 receptor antagonist (ra)$^{-/-}$ mice. Antibody production against sheep red blood cells was significantly reduced in IL-1$\alpha/\beta^{-/-}$ as well as IL-1$\beta^{-/-}$ mice, partially in IL-1$\alpha^{-/-}$ mice, and enhanced in IL-1ra$^{-/-}$ mice. The intrinsic functions of T, B, and antigen-presenting cells (APCs) were normal in these mice. IL-1$\alpha/\beta^{-/-}$ APCs did not fully activate DO11.10 T cells with OVA, while IL-1ra$^{-/-}$ APCs enhanced the reaction. The effect of IL-1 was CD28-CD80/CD86-independent, because the effect of IL-1-deficiency was also observed in the presence of CTLA4 Ig. The expression of CD40L and OX40 on T cells was greatly affected in these reactions. Furthermore, the impairment of proliferation and OVA-specific Ab production of IL-1$\alpha/\beta^{-/-}$ B cells cocultured with mitomycin C-treated DO11.10 T cells could be reconstituted by the addition of agonistic anti-CD40 mAb. These observations suggest that IL-1 enhances Ab production and T cell-priming by stimulating CD40L and OX40 expression on T cells upon interaction with APC in humoral immune response.

01014

HEAT SHOCK OF APC: EXPRESSION OF DIFFERENT PATTERNS OF CYTOKINES BY NAIVE AND MEMORY T CELLS.

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Recently we have found that moderate heat shock of APC results in a fast decrease in a levels of mRNA for costimulatory ligands CD80 and CD86. In this work we have shown that this effect is accompanied by profound decrease in a surface expression of B7-1 protein. We investigated the outcomes of this effect on responses of naive cells and *in vivo* primed memory cells specific to H-2K^b molecule with respect to their proliferation and production of different cytokines and costimulatory ligands *in vitro*. Primary proliferative response to the alloantigen was shown not to develop when the allogenic antigen-presenting cells were subjected to a severe (45°C, 1 h) or moderate (42°C, 30 min) heat shock. On the contrary, the long-lived memory CD8$^+$ T-cells induced in the course of the primary *in vivo* response were capable of proliferation in response to heat-treated allogenic stimulators carrying the same immunizing antigen. We investigated the expression of a number of cytokines and costimulatory molecules in a mixed cultures of splenocytes from intact and immune animals in response to stimuli provided by the allogenic stimulators subjected to an irradiation or severe heat shock. This number included: GAPD (control), IL-2, IL-10, TGFβ_1, IFN-γ, TNFα, CD40L and FasL. mRNA for these markers and cytokines was estimated in 6 day cultures by the PCR of cDNA libraries obtained using Superscript. The cultures containing naive T lymphocytes of B10.D2(R101) mice (K^{d}I^{d}D^b) and irradiated stimulators of the C57BL/10 mice (K^{b}I^{b}D^b) expressed all these markers and cytokines. Cultures containing a naive T cells with heat shocked stimulator cells expressed only GAPD, TGFβ_1 and FasL. The patterns of expression of these markers in memory cells, stimulated with irradiated and heat-shocked stimulators were similar and besides GAPD included: IL-10, TGFβ_1, IFN-γ, TNFα and FasL. It is interesting that in cultures containing a memory cells specific to H-2K^b production IL-2 and CD40L was not observed, though memory cells successfully proliferated *in vitro*. Thus, the proliferation of memory cells in response to allogenic stimulators subjected to a heat shock, can be dependent from others cytokines than IL-2. According to the data of the literature, the most probable cytokines are TGFβ_1 and IL-10.

01024

Role of cytokines in development of atopic inflamation in recidivating croup in children

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Immunologic mechanisms of development of chronic recidivating inflamation were studied in 71 children with recidivating croup (3-35 recidives). IgE level was investigated with method of hard-phase IFA. Membraneimmunofluorescence was used for determination of activity level of CD4+, CD8+ and natural killers (activated NK CD16+8+), which have important role in elimination of disease agents from an organism. Interferon status was investigated according to method, suggested by S.S. Grigoryan (1988). Migration and esterase activity of macrophages was evaluated with skin aseptic reaction (SAR) according to Rebuk J.W. (1955).

IgE level was increased significantly upto 325 33.7 IU/ml with norm of 75 2.6 IU/ml (20-130 IU/ml) with background of significant decrease of CD4+ (20.5 1.7% with 37 1.6% in norm) and CD8+ (15 2.7 with 29 2.1% in norm). Activated NK CD16+8+ were decreased moderately (1.1 0.5% with 1.4 0.2% in norm). There was as well decreasing of αIFN (11.7 2.3), γIFN (6.3 1.4) and serum IFN (2 0.7) with 17.6 1.3, 15.4 1.2 and 6.2 0.1 in norm correspondingly. Chaemotaxis of macrophages in the SAR zone was decreased to 8.5 2.2% ($p<0.001$) with 75 1.1% in norm. Esterase activity was detected in 4 1.9 ($p<0.001$) with 16 1.6 macrophages with specific staining in norm. Significant production of collagene with simultaneous accumulation of fibroblasts and neutrophiles was found in the SAR zone at the same time.

Thus, decreasing of physiological inhibiting effect of γIFN on synthesis of IgE takes place in children with recidivating croup. That is result of predomination of T x 2 subpopulations, which are responsible for differentiation of B-lymphocytes into IgE-producing plasmatic cells, which results in hyperproduction of IgE, which level determines largely severity of the atopic inflamation.

01015

PROTHYMOSIN-α PARTICIPATES IN REGULATION OF THE INTERACTION OF IMMUNE, NERVOUS AND ENDOCRINE SYSTEMS

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Immunostimulating action of prothymosin-α (ProTα) on the maturity and differentiation of T-lymphocytes from thymus and bone marrow had been found. Pro Tα was shown to be a factor which stimulated effectively recovery of T-cell immunity and took part in glucocorticoid functioning in thymocyte apoptosis demonstrating the interaction of immune and endocrine systems. ProTα was stated to demonstrate manifested antiviral activity. ProTα demonstrated its adaptogenic functions intensificating short-time and consolidated memory and endurance of experimental animals under the extreme conditions. As a whole, the data obtained are being the first experimental evidence of universality of ProTα action in the host organism - participation in regulation of the functions of immune, nervous and endocrine systems.

01016

INFLUENCE OF HYMORAL FACTOR OF DN ($CD4^-8^-$) THYMOCYTES ON THE MIGRATING PROCESS OF EARLY PRECURSORS OF BONE MARROW

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In the previous work we have shown that mature cortisone-resistant double negative cells which have gone the positive selection and have the phenotype $CD4^-8^-TCR^+$ are able to synthesize spontaneously a chemotactic factor. This factor takes part in the regulation of the migration of bone marrow stem cells to periphery. In the experimental system this fact takes place in various distress situations in vivo: sublethally irradiation and various immunodeficient conditions including hereditary illness. It is possible that this fact takes place in normal ontogenesis, realizing the regulatory connection between thymus and bone marrow. On the next step we have tried to define the target cells of the studying factor . We have divided the heterogeneous population of bone marrow cells with the help of percoll with the density 1.06; 1.07; 1.08; 1.09. Using the in vitro migrating test we have defined that the target cells of the studying factor are located in the 1.06; 1.07 fractions. The morphological analysis have shown that these fractions consist of low differentiated cell elements, different precursors of lymphoid and hematopoietic lines. The main population of the 1.08; 1.09 fractions consist of the mature granulocytes.

01017

VEGF INCREASES SUSCEPTIBILITY OF THYMOCYTES TO APOPTOSIS

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Thymic involution accompanies many rodent and human tumors, but its mechanism remains unknown. We have previously shown enhanced spontaneous apoptosis of thymocytes from tumor-bearing animals (Kisseleva et al., 1998). Here, we investigate a possible role of Vascular Endothelial Growth Factor (VEGF) in this process. VEGF is produced by almost all tumor cells and is required for neovascularization. Continuous 4-week infusion of VEGF via s.c. osmotic pumps into BALB/c mice resulted in the development of thymic involution very similar to that observed in tumor-bearing animals. To evaluate VEGF effect of apoptosis of thymocytes mice were exposed to VEGF for one week, before any morphological or subpopulational changes in thymus started. Spontaneous and dexamethasone-inducible apoptosis of thymocytes was studied after 3- hr culture *in vitro* using Annexin V staining. Susceptibility to dexamethasone-induced apoptosis increased 1.3 times in mice with VEGF pumps in comparison to PBS treated controls. Then, the direct effect of VEGF on the apoptosis of thymocytes from intact animals was evaluated. Thymocytes incubated during 24 hr in the presence of 50 ng/ml VEGF showed increased spontaneous apoptosis as measured by sub-G_1 peak after PI staining (38.2±1.0% compared to 33.8±0.7% in control, $p<0,05$) and confirmed by Annexin V binding. Cultivation of thymocytes during 72 hr in the presence of 50-100 ng/ml VEGF revealed its immunomodulatory effect on proliferation in response to ConA. This was the first attempt to evaluate the direct effect of VEGF on apoptosis and proliferation of thymocytes. These data indicate the possible role of angiogenic factors in the impairment of T-lymphocyte maturation and differentiation during tumor growth. The work was supported by NATO Collaborative Linkage grant SA(LST.CLG 975197) and Russian RFBR grant N.00-904-49430.

01018

Significance of Interferon-γ in regulation of immune response in children with often and prolonged diseases

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Immunologic mechanisms of development of T-cell link of immunity was studied in 89 children of 1 to 3 years old – 11 with recidivating croup (RC), 40 with recidivating obstructive bronchitis (ROB) and 38 with often and prolonged diseases (OPD). Lymphocites subpopulations CD4+, CD8+, cytotoxic cells NK (CD16+), activated NK (CD16+8+), ILR2 were investigated using membrane immunofluorescence. Interferon status was determined according to S.S. Grigoryan (1988).
CD4+ level was decreased significantly in all children (22±1.9% in RC, 26.7 1.8% in ROB, 28.4 1.5% in OPD with 37 1.6% in normal children) with simultaneous decreasing of CD8+ (14.6 2.6, 16.5 2.1, 21.7 3.0 and 29 2.15% correspondingly). That caused decreasing of potential the link, which induces antibodygenesis (KThs in RC - 1.51 0.03, ROB - 1.6 0.05, OPD – 1.3 0.02 with 2.25 in normal children), to supressing link of regulation and, consequently, to antiviral protection too. ILR2 index was decreased as well (RC – 6.1 1.7%, ROB – 7.3 1.1%, OPD - 7.8 1.4% with 11 0.8% in normal children). γIFN level was below normal index by 2.5 times in children with ROB and OPD and by 3.4 times in children with RC (RC – 4.5 0.8, ROB - 6.1 1.2, OPD – 4.6 0.7 with 15.4 1.2 in normal children). Indices of NK (CD16+) and activated NK (CD16+8+) were decreased moderately.
Thus revealed correlative connection between γIFN level and cytotoxic activity of T-lymphocytes is an evidence of uncompleted differentiation of cells: deficiency of transition of T x 0 into T x 1 and excess of one into T x 2 due to affection of them by different inhibiting factors.

01019

FUNCTIONAL AND PHENOTYPIC IDENTIFICATION OF IL-13 AND IFN-γ PRODUCING HUMAN NK CELL SUBSETS.

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Distinct NK cell subsets produce type 1 (e.g. IFN-γ) and type 2 (e.g. IL-13) cytokines exclusive of each other, and IL-13 producing cells are detectable in freshly isolated bronchoalveolar lavages from allergic asthmatic patients, suggesting a possible role for these cells in pathological conditions. To better define the IL-13 producing (IL-13$^+$) subset, we have analyzed the regulation of its proliferation/differentiation, surface phenotype, and cytokine production in response to different stimuli (phorbol ester and Ca^{2+} ionophore, contact with target cells). Cultures of primary peripheral blood lymphocytes (PBL) with IL-4 or IL-12 in the presence of IL-2 consistently resulted in increased and decreased numbers of IL-13$^+$ NK cells, respectively. The number and percentage of this subset was also decreased in cultures with IFN-γ, independently from IL-12. Multiple color (surface and intracellular) flow cytofluorimetric analysis indicated that the phenotype of the IL-13$^+$ NK cells is distinct from that of the IFN-γ$^+$ ones. IL-13 production is restricted to CD8$^-$/CD56$^{-/lo}$ (CD161$^+$) NK cells, while IFN-γ is produced by CD8$^+$, CD8$^-$, CD56^{lo} and CD56^{hi} NK cells. Additionally, IL-13 production is not restricted to CD25$^+$ NK cells, nor a correlation exists between its production and NK cell proliferation, unlike what reported for IL-5 production, and the percentage of IL-13$^+$ NK cells correlates with that of IL-12Rβ2$^-$ cells. Clonal analysis revealed that both IFN-γ$^+$ and IL-13$^+$ NK cells can be derived from a single NK cell, but that NK cells committed to IFN-γ production can not be induced to produce IL-13, implying possible irreversible differentiation of an uncommitted progenitor.

01020

CYTOKINE-LIKE PROPERTIES OF PEPTIDE THYMOMIMETICS

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Peptide thymomimetics produced in the process of limited proteolysis out of precursor macromolecules (cytokines, growth and thymic factors, immunoglobulins and other proteins) are important in regulating cellular immunity and inflammation processes. The basic mechanism of peptide thymomimetic action is associated with maintaining a certain correlation of T-cell population composition at various stages of development. Natural peptide thymomimetics were isolated from the thymus of calves by mild acid extraction. An immunomodulatory molecule - Glu-Trp – was isolated by RP-HPLC from a peptide complex and synthesised. Both natural and synthetic peptides activated T-cell differentiation and cytokine excretion (IL-2, IFN) of lymphocytes. Studied were the biological properties of a new thymomimetic dipeptide Lys-Glu, which can be found in such polypeptides as α_1 thymosin, β_7-β_{10} thymosins, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, and IFN-α. It restores the immune response, DNA, RNA and protein synthesis in T-lymphocytes of old and immunodepressed animals. Long-term administration of the dipeptide increased the animal life span and significantly decreased spontaneous tumour incidence. The dipeptide in concentrations from 1 ng/ml to 100 ng/ml modulated T-cell growth and lymphoid-mediated angiogenesis. In concentrations from 50 pg/ml to 100 ng/ml the dipeptide stimulated IL-2 mRNA synthesis in the spleen lymphocytes of mice. Besides, the dipeptide activated neutrophil chemotaxis and phagocytosis. The obtained results show that Glu-Trp and Lys-Glu dipeptide belongs to a new group of transmitters playing an important role in the regulation of innate immunity.

01013

MONOCYTIC MIP-1α PRODUCTION INDUCED BY COGNATE INTERACTION WITH T CELLS IS DIFFERENTIALLY DEPENDENT ON NFκB DEPENDENT

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While the mechanisms involved in macrophage production of chemokines are not yet understood, chemokines have been demonstrated to play a role in the pathophysiology of inflammatory disease. We examined the mechanisms involved in the production of the CC chemokine MIP-1α in rheumatoid arthritis synovial tissue and role NFκB plays in human monocyte-T cell cognate MIP-α production. T cells were activated either with anti-CD3 antibody, with a combination of cytokines (IL-2, IL-6 & TNFα), or isolated from RA synovial joints, fixed and co-cultured with macrophages. MIP-1α secretion by macrophages co-cultured with all three T cell populations resulted in a significant up-regulation of macrophage MIP-1α in a cognate dependent manner. Next we examined whether T cells-macrophage co-culture induced MIP-1α in a NF-κB dependent fashion by using a viral vector expressing the inhibitor (AdvIκBα). Over-expression of IκBα significantly reduced monocytic MIP-1α production by 76% in MCSF treated macrophages co-cultured with anti-CD3 activated T cells in comparison with Ad0 or uninfected macrophages. In contrast, over-expression of IκBα had little effect on MIP-1α secretion in MCSF macrophages when stimulated by cytokine activated or RA T cells infected with AdvIκBα, Ad0 or uninfected. However, when TNFα secretion was examined, it was the cytokine stimulated and RA T cells that induced TNFα production which was abrogated by blockage of NFκB. This work demonstrates that whereas cytokine/chemokine production may be dependent upon cognate interaction with a T cell component, different forms of T cell stimulation differentially dependent on NFκB for macrophage stimulation.

01023

ALTERATION OF CYTOKINE LEVELS AND SPECIFIC IMMUNE RESPONSES IN ALCOHOL CONSUMING MICE

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Excessive, prolonged alcohol consumption impairs induction of cell-mediated immune responses and elevates serum antibody levels. These alterations to both innate and adaptive immune responses account for the increased frequency and severity of infections seen in alcoholics. Ethanol's effect is most pronounced during the cognitive phase of the immune response resulting in a profound down regulation of the Th1 cytokines, IL-12 and IFN-γ, and subsequent impairment of delayed hypersensitivity responses. Alcohol up-regulates IL-4 and enhances antigen-specific Th-2 mediated antibody responses. Here we show that APC from alcohol-consuming BALB/c mice when co-cultured with T cells derived from non-alcohol-consuming syngeneic DO11.10 transgenic mice down regulates the OVA-specific Th1 response. Although no alteration is seen in OVA-specific T cell proliferation or IL-2 levels, IL-12 and IFN-γ ELISA responses are profoundly down regulated. Analysis of intracellular staining by flow cytometry confirms that alcohol does not alter the number of antigen-specific (KJ1-26+) CD4+ IL-2 producing cells, yet the number of CD4+, KJ1-26+ IFN-γ producing cells is decreased. Conversely, transgenic T cell derived from alcohol-consuming DO11.10 mice when co-cultured with APC from non-alcohol consuming BALB/c mice show normal Th1 cytokine responses. Our data indicate that alcohol alters APC function with a profound affect upon the Th1/Th2 cytokine and functional response patterns.

01009

IMMUNE STATUS IN NEWBORNS AFTER INTERFERON TRERAPY OF PREGNANT WOMEN WITH CYTOMEGALOVIRUS AND HERPES SIMPLEX VIRUS INFECTION

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Cell immunity were analysed in 52 newborns during the first day of their life, who were born by mothers infected with cytomegalovirus (CMV) and herpes simplex virus (HSV) and treated with Viferon in the complex therapy against these infections. Comparative group included 44 newborns from mothers with the same infections, who however did not receive Viferon in the complex therapy.
Viferon is a complex preparation, which includes human recombinant interferon-α2b, membrane-stabilising substances and cocoa oil as a base. Viferon therapy of pregnant women was carried out from 28th week of gestation to birth of a child.
Analysis of the phenotypic characteristics of lymphocytes of women, treated with Viferon, showed increase in relative number of T-lymphocytes, CD8+ lymphocytes, growth of decreased and reduction of increased values of immunoregulating index, increase in relative number of natural killers.
Viferon treatment of pregnant women was shown to result in normalization of phenotypic characteristics of lymphocytes of newborns, in increasing of HLA DR antigen level and inhibition of antigen stimulation, reducing number of NK and activated NK.
Thus treatment with Viferon of pregnant women with CMV and HSV infections in the 3rd trimester of gestation contributes to favorable course of pregnancy, enhances antiviral immunity, reducing frequency of intrauterine infections. Treatment with Viferon of the infected mothers decreases antigenic stimulation of fetal immunity and normalizes immunological indices of newborns, contributing to development of physiological immune response in case of intrauterine infection.

01022

PROLIFERATIVE RESPONSE TO CYTOKINES AND PHENOTYPE PROFILE OF LONG-LIVED THYMOCYTES

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In previously studies we established that thymocytes surviving in vitro after gamma-irradiation of 50 Gy or after culture without cytokines for 20 days can be activated to proliferate by an apparently novel thymocyte growth factor (THGF), which was produced by a $CD4^-CD8^-$ thymic cell line (Shichkin 1990, 1992). In the current study we irradiated (50 Gy) thymocytes and compared them to cultured, non-irradiated thymocytes for surface markers and their proliferative response to cytokines. Cells were analyzed on day 5, 10, 20 and 90 of culture. Number of viable thymocytes in both irradiated and non-irradiated cell cultures progressively decreased to 10-15% by day 20. The proportion of viable cells remained near 95% up to day 90. On day 10, most viable cells were $CD4^+CD8^+$ and $CD25^+CD44^-$. Minor populations of $CD4^-CD8^-$, $CD4^+CD8^-$, $CD8^+$ $CD4^-$ and $CD25^-CD44^-$, $CD44^+CD25^+$, $CD44^+CD25^-$ were observed. However at day 90, most cells were $CD4^-$ $CD8^-$ and $CD25^-CD44^-$. Irradiated thymocytes slowly proliferated without exogenously added cytokines and by 90 day did not respond to IL-2, IL-3, IL-4, IL-7, IL-9, SCF, GM-CSF alone or in combinations. However, up until day 20, cells remained capable of proliferating in response to several cytokines. In non-irradiated cultures this response was more vigorous. Our data suggest that thymocytes cultured for prolonged periods may maintain their survival and proliferation by production of autocrine factors that do not correspond to previously identified cytokines.

Cytokines in sepsis and toxic shock

02003

MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) : A CENTRAL MEDIATOR OF INNATE IMMUNE RESPONSES AND SEPTIC SHOCK

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Discovered in the early 1960s as a T-cell cytokine, MIF has emerged recently to be an important mediator of the innate immune system. MIF is released by numerous cells, including monocytes/macrophages, T cells, B cells, endocrine cells (pituitary and adrenal cells) and epithelial cells in response to infection and stress. Bacteria, microbial toxins (LPS, TSST-1, SPEA) and cytokines (TNFα, IFNγ) were found to be powerful inducers of MIF secretion by macrophages (*J.Exp.Med. 1994;179:1895*). MIF stimulates the expression of pro-inflammatory mediators by immune cells and functions to counter-balance the anti-inflammatory effects of glucocorticoids (*Nature 1995;377:68*). Like TNF and IL-1, MIF plays an important role in septic shock. rMIF was found to exacerbate lethal endotoxemia or bacterial sepsis when co-injected with LPS or *E. coli* in mice. Conversely, mice treated with anti-MIF antibodies or MIF knockout mice were protected from shock induced by LPS, staphylococcal exotoxins or bacterial peritonitis, even when anti-MIF therapy was started after the onset of infection (*Nature 1993;365:756; J.Exp.Med 1999;189:341; Nat.Med.2000;6:164*). Recently, MIF was found to regulate host responses to endotoxin and Gram-negative bacteria by modulating the expression of Toll-like receptor 4. Given the central place of MIF in host responses to infection, pharmacological modulation of MIF production or neutralization of MIF activity could have broad clinical applications, and may offer new treatment options for patients with severe sepsis and septic shock.

02001

SUPERANTIGEN ANTAGONIST BLOCKS Th1 CYTOKINE GENE INDUCTION AND LETHAL SHOCK

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Bacterial superantigens are among the most lethal of toxins. By activating up to 50% of T cells, they trigger an excessive cellular immune response leading to toxic shock. The high cytokine levels produced in response to superantigens have obstructed efforts to block downstream phenomena in the toxicity cascade set off by a pyrogenic toxin, for example, with monoclonal antibodies against TNF. We have designed a peptide antagonist that inhibits superantigen-induced expression of human genes for IL-2, IFN-γ and TNF-β, Th1 cytokines that mediate shock. At only 100-fold molar excess over superantigen, this peptide blocks induction of the corresponding mRNAs by a superantigen, yet it fails to inhibit such induction by a conventional antigen such as tuberculin PPD. Hence, the antagonist exhibits specificity for superantigens. Induction of IL-2, IFN-γ and TNF-β mRNA in human PBMC by different superantigens was also inhibited by antibodies raised against the peptide. The peptide antagonist shows homology to a β-strand-hinge-α-helix domain that is structurally conserved in superantigens produced by *Staphylococcus aureus* and *Streptococcus pyogenes*, yet remote from known binding sites for the major histocompatibility class II molecule and T-cell receptor. Superantigens depend for T-cell activation on this new domain. The peptide protected mice against lethal challenge with staphylococcal and streptococcal superantigens. Moreover, it rescued mice undergoing toxic shock. Surviving mice rapidly developed protective antibodies to superantigen that rendered them resistant to further lethal challenges, even with different superantigens. Thus, the lethal effect of superantigens can be blocked with a peptide antagonist that inhibits their action at the top of the toxicity cascade, before activation of T cells and induction of Th1 cytokine genes takes place.

Arad, G., Levy, R. Hillman, D. and Kaempfer, R. (2000). Superantigen antagonist protects against lethal shock and defines a new domain for T-cell activation. **Nature Medicine** 6, 414-421.

02002

Protection against TNF-induced lethal shock by soluble guanylate cyclase inhibition requires functional Nitric Oxide Synthase-2

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Tumour Necrosis Factor (TNF) exerts a remarkable antitumour activity but is also a cardinal mediator of inflammatory diseases and shock. Its hemodynamic effects are dose limiting for its use in systemic anti-cancer treatment. Hypotension and shock observed in sepsis, SIRS and cytokine-based cancer treatment are the consequence of excessive Nitric Oxide (NO) production and subsequent soluble guanylate cyclase (sGC) mediated vascular smooth muscle cell relaxation. We found that while NO synthase (NOS) inhibitors enhanced rather than inhibited the toxicity, inhibitors of the activation of sGC protected against TNF-induced lethality, bradycardia and hypotension. Most importantly, these inhibitors did not interfere with the antitumour activity of TNF We furthermore observed that no protection against TNF toxicity can be obtained in the absence of NO, when NOS inhibitors or NOS-2 deficient mice are used. These data imply that NO produced by the inducible NOS-2 and not the endothelial NOS-3 is an endogenous protective molecule indispensable to survive a challenge with TNF and exerting this beneficial effect via sGC independent mechanisms. Therefore, we suggest that sGC is a more useful target than NOS-2 to prevent TNF toxicity in TNF mediated diseases or to widen the systemic antitumour potential of TNF.

02005

N-ACETYLCYSTEINE (NAC) AUGMENTS MIGRATION OF NEUTROPHILS TO THE SITE OF INFECTION BUT NOT THAT TO THE LUNG: GLUTATHIONE MODULATION OF NATURAL IMMUNITY VERSUS ARDS IN SEPSIS.

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Migration of neutrophils (PMN) has a dual role in infection: it represents a first line of defense against bacterial infection, but is also a component of sepsis-induced ARDS. Glutathione (GSH) also has somewhat opposite effects in immunity: it is antiinflammatory, inhibiting production of several cytokines, but it is essential for several immune functions.
Since GSH is lower in sepsis and in conditions associated with increased susceptibility to infections (AIDS, alcoholism) we investigated the effect of GSH depletion (with buthionine sulfoximine) or repletion (with NAC) on PMN migration in a model of peritoneal sepsis (cecal ligation and puncture, CLP) in mice.
GSH depletion inhibited peritoneal PMN infiltration in CLP, and consequently increased bacterial colonies and worsened survival. Ex vivo experiments showed that PMN from GSH-depleted mice do not have lower migration capacity, and that there is no defective production of overall chemotactic activity in the peritoneal exudate.
On the contrary, lung PMN infiltrate observed with CLP was augmented by GSH depletion. Oral administration of NAC to CLP mice augmented PMN infiltration in the peritoneum, but not in the lung, decreased bacterial colonies and improved survival. These data show that GSH differentially affects leukocyte migration and has a complex role in natural immunity and inflammation.

02006

ALTERED SENSITIVITY TO TNF-INDUCED TOXICITY IN METALLOTHIONEIN (-I AND -II) NULL MICE AND MT-I TRANSGENIC MICE.

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Leptin is a cytokine primarily produced by adipocytes which provides a negative feedback signal to hypothalamic regions involved in satiety and the body's energy homeostasis. Characteristic for many cytokines, leptin is also pleiotropic with peripheral effects on endocrine balances, haematopoiesis, inflammation, angiogenesis and fertility.

Previously, we showed that leptin is involved in the protection against Tumour Necrosis Factor (TNF) induced toxicity. Using RDA analysis in PC12 cells stably expressing the leptin receptor, we were able to identify a number of leptin induced genes. One of these genes encodes Metallothionein 2 (MT-2), which is a member of a family of highly conserved metal-binding proteins that are reported to function in detoxification of heavy metals, in Zn and Cu homeostasis, scavenging of free radicals, and acute phase response. Metallothionein was of particular interest since we were able to show modulation of metallothionein mRNA and protein levels by leptin both in vitro (PC12 cells) and in vivo (using wildtype and leptin deficient ob/ob mice).

In the present study, MT-1 transgenic mice and mice with null mutations for MT-1 and MT-2 were challenged with different doses of TNF intravenously. At different time points blood serum samples were taken for IL-6 and NO measurement. We demonstrate that mortality was increased by TNF in the group of Metallothionein-I transgenic mice versus controls. In line with this observation we also show that Metallothionein null mice are more resistant to TNF induced toxicity.

02004

AUGMENTED NO PRODUCTION AND HIGHER RESISTANCE TO ENDOTOXEMIA IN TRANSGENIC MICE OVEREXPRESSING TSG-14/PTX3

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Tumor necrosis factor-stimulated gene 14 (TSG-14)/PTX3 was originally identified as a TNFα and IL-1β stimulated gene from normal human foreskin fibroblasts and vascular endothelial cells, respectively. TSG-14 gene encodes a 42 kDa secreted glycoprotein with a carboxy-terminal half that shares homology with the entire sequence of C-reactive protein (CRP) and serum amyloid P component (SAP) acute-phase proteins of the pentraxins family. Some experimental evidences suggests that TSG-14 plays a role in inflammation, yet its function and mechanism of action remains unclear. We have generated transgenic mice that overexpress the TSG-14 gene under the control of its own promoter. Two transgenic lineages were derived: Tg2 and Tg4, carrying two and four copies of the transgene, respectively. Macrophages derived from these transgenic mice produce higher amounts of nitric oxide in response to IFNγ and LPS stimuli as compared to macrophage from wild type animals. In addition, TSG-14 transgenic mice were found to be resistant to the lethal action of LPS. The survival probability for each group when injected with LPS differed significantly (p=0.01): 72%, 52.1% and 34.2% for Tg2, Tg4 and wt mice respectively. These results are the first *in vivo* evidence of the involvement of TSG-14 in the inflammatory process.

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02011

IMPROVED SURVIVAL OF TNF-DEFICIENT MICE DURING THE ZYMOSAN-INDUCED MULTIPLE ORGAN DYSFUNCTION SYNDROME

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Tumor necrosis factor(TNF) is believed to be a crucial mediator in systemic inflammation and the multiple organ dysfunction syndrome (MODS). Previous studies in our laboratory with a murine model have shown that interventions directed at TNF could mitigate the symptoms of zymosan-induced MODS. The present study aimed to investigate the course of MODS in the absence of TNF.
24 TNF/LT- knockout (-/-) mice and 25 wildtype (+/+) mice received 40 µg LPS ip, followed by zymosan at a dose of 1 mg/g body weight 6 days later (day 0). Survival, body weight and body temperature were monitored. At day 22 10 -/- mice and 5 +/+ mice were killed and organs were collected and weighed. The remaining animals were followed until day 48, when all were killed and their organs harvested for histology. In all animals zymosan induced an acute sterile peritonitis phase followed by an apparent recovery. From day 8 onwards the +/+ mice entered a third - MODS-like - phase, characterized by loss of body weight, decreased body temperature and significant mortality. At day 22, survival in the -/- mice was 92%, which was significantly ($p<0.05$) higher than in the +/+ mice (60%). In addition, average body temperature and average relative (vs. weight at day 0) body weight were 33.3°C and 84%, respectively in the +/+ mice. Data for the -/- mice were 35.9°C and 100%, respectively ($p<0.05$). However, at this time point, both the +/+ mice and the -/- mice showed significant and similar organ damage, indicated by an increase in absolute and relative (vs body weight) weight of lung, spleen and liver (the last organ only in the -/- mice). Moreover, at day 48, histopathological examination of liver, lungs, spleen and kidneys from the surviving animals showed a similar degree of microscopic damage in both the -/- and +/+ animals. TNF-deficient mice exhibit significantly improved survival and clinical condition during zymosan-induced MODS. Still, surviving animals from both groups eventually display a similar degree of organ damage. Thus, in this murine model, the absence of TNF does not completely protect against MODS.

02010

BENZNIDAZOLE, A DRUG USED IN CHAGAS DISEASE INHIBITS EXPERIMENTAL SEPTIC SHOCK

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Benznidazole (BZL) is a compound which has extensively been used for the treatment of Chagas´disease a protozoan disease caused by *Trypanosoma cruzi*. We have shown that BZL downregulated the synthesis of nitric oxide (NO) and cytokine synthesis by LPS and/ or IFN-γ-stimulated murine macrophages (Revelli et al 1999 Clinical Exp. Immunol.) Molecular studies indicated, that BZL affected the inducible NO synthase (iNOS) gene expression. To analyse whether the latter effect also extended to the *in vivo* situation, systemic treatment with BZL was applied to mice undergoing LPS-induced experimental endotoxemia. C57BL/6 mice were challenged intraperitoneally with LPS at a single dose of 200 µg/mouse, half of them also receiving BZL treatment. BZL was given orally 18 and 2 h before LPS challenge as well as 24 h following endotoxemia induction, at a dose 400 mg/kg/bw each. Livers were removed at 4 and 24 h post-induction, for RNA extraction, being followed by cDNA synthesis and PCR amplification. Semiquantification of iNOS gene expression performed after coamplification and normalization with the β actin internal control indicated that BZL caused a 70% inhibition of iNOS gene expression by LPS-treated animals at both time point evaluations. Measurements at 90 min and 4 h post-induction indicated that BZL treatment resulted in significant reductions of TNF-α serum levels respect to untreated mice (90 min, mean ± sem pg/ml: BZL 2723±310, untreated 5064±1103; 4-h: BZL 235±27, untreated 366±58). Conversely, IL-10 appeared to be sligly increased in the BZL-treated group. Control mice undergoing BZL treatment had no measurable cytokine amounts in their sera. Animals injected with two LPS separated doses (200 µg each, with a 4-h interval) and receiving the same BZL treatment, before and after the respective first and second LPS challenge, had a decreased mortality (3/10) if compared to their untreated counterparts (8/9, $p<0.02$). Present effects may broaden the potential usefulness of BZL in situations accompanied by an excessive inflammatory response or in septic shock.

02009

PROGRESSION TO A PRO-INFLAMMATORY STATE FOLLOWED BY REGRESSION TO AN ANTI-INFLAMMATORY STATE AFTER A SINGLE STIMULUS IN AN *EX VIVO* SYSTEM

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Following initiation the inflammatory response evolves through phases with synthesis of pro-inflammatory cytokines followed by their naturally occurring inhibitors. We examined the kinetics of cytokine/inhibitor release to determine the natural history of the inflammatory response. Blood was obtained from 7 normal donors and stimulated with 1 μg/ml of LPS. Samples were harvested between 0 to 80 hrs and plasma levels of TNF, TNF-SR Type I &II, IL-1, and IL-1ra, were measured by ELISA and/or bioassay. Biologically active and total TNF peaked between 6 and 8 hrs post LPS at levels of 10.2±2 ng/ml and 18.2±2.3 ng/ml followed by a decline to levels of .2 ± .07 and 1.8±.4 ng/ml at 80hrs respectively. Total TNF appeared at levels about 2X those of biologically active TNF TNF-SR I&II both increased from baseline levels of 145±41 and 660±17pg/ml to 453±107 and 3150±421pg/ml at 22hrs respectively, then remained constant until 80hrs. Similar to TNF, IL-1 was rapidly generated and reached 23.2±4.2ng/ml at 8hrs while IL-1ra achieved peak levels of 33.5±7.5ng/ml at 22hrs. Both remained constant up to 80hrs The "inflammatory state" was established by calculating the molar ratio of the pro-inflammatory cytokines and their inhibitors to document the progression from an anti(AI) to pro-inflammatory(PI) state. A high ratio indicates more inhibitor compared to cytokine, indicating a less inflammatory state. The molar ratio of IL-1ra:IL-1 was 33± 22 at 0hr (i.e. prior to any stimulus), achieved a nadir at 1.1±0.4 at 8hr and remained at this ratio until 80hrs. For TNF (TNF-SR I+II/TNF) the ratio was 6.1±1.4 at 0hr, decreased to 0.06±0.09 at 6hrs followed by a return to AI with a ratio of 12.5±4.6 at 80hr. Therefore, in the normal condition, the inhibitors are in molar excess compared to the cytokines. Following a stimulus in the there is progression to and from a pro-inflammatory state within 8 hours. Examining the IL-1 system, the blood became pro-flammatory by 8 hours and remained so up to 80 hours. However, the TNF system regressed back to AI which was mediated by the clearance of TNF and not by the increased release of TNF-SR.

02008

INCREASED SUSCEPTIBILITY TO LIPOPOLYSACCHARIDE OF IL-6 TRANSGENIC MICE

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Markedly elevated circulating levels of IL-6 are found in experimental models of sepsis and septic chock. In humans with sepsis and septic shock, IL-6 levels correlates with clinical scores. However, the role of IL-6 is still controversial, with some data pointing to a protective role and other to a detrimental one. In this study we have evaluated the outcome of LPS-induced shock in IL-6 transgenic mice. NSE/hIL-6 transgenic mice, with ng/ml levels of circulating hIL-6, were used. NSE/hIL-6 and wild-type littermates were injected i.p. with E. Coli LPS at 10, 20, 40, 60 and 100 μg/gr of body weight. NSE/hIL-6 mice showed a marked increase in the mortality at doses that were not lethal in the wild-type (WT) littermates.

	Mortality (dead/total)				
LPS	10 μg/gr	20 μg/gr	40 μg/gr	60 μg/gr	100 μg/gr
WT	0/7	0/7	0/10	1/7	5/5
NSE/hIL-6	2/6	6/7	10/11	13/13	6/6
p value	>0.1	0.002	<0.001	<0.001	>0.1

Similar increased susceptibility was also observed when sublethal doses of LPS from S. Enteritidis or from K. Pneumoniae were used. In addition, NSE/hIL-6 mice showed also increased mortality to the administration of pertussis toxin ($p<0.001$). Evaluation of TNF-α production showed that 90 min. after E.Coli LPS administration (40 μg/gr) NSE/hIL-6 transgenic mice had TNF-α levels (11,9 ± 14,4 ng/ml) higher than those of wild-type mice (4.8 ± 3.4 ng/ml), but the difference was not statistically significant. On the contrary, a significant difference was found 180 minutes after LPS administration (NSE/hIL-6: 15,1 ± 19,8 ng/ml; wild-type: 0,9 ± 1,2 ng/ml; $p<0.01$). Our data show that the human IL-6 transgenic mice have increased susceptibility to LPS, associated with a higher and more sustained production of TNF-α.

02007

Suppression of TNF-α by the Novel Analogs of Imiquimod

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Introduction We show that imidazoquinoline derivative BI-345 that is structurally related to "imiquimod" has novel tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) suppressing activity in human peripheral blood mononuclear cells and in mice. We have originated not only potent IFN-α inducer BI-087 but also TNF-α/IL-1β suppressor BI-345 through the modification of imiquimod.

Method Peripheral blood mononuclear cells (PBMCs) from healthy volunteers were incubated with test compounds at 37°C in RPMI1640 based medium and in 5 % CO_2 atmosphere for 30 minutes. The cells were treated with or without 1 μg/mL lipopolysaccharide (LPS: *E. coli*, 0111:B4, Sigma) and were incubated further for 16 hours. Levels of TNF-α, IL-1β and IFN-α in the culture supernatant were determined by ELISA. BALB/c mice were orally administered the test compound or vehicle 120 min before challenge with intraperitoneal injection of 25 μg of LPS (*E. coli*, 055:B5; Sigma) After 120 min, blood was collected by exsanguination, and plasma samples were used to measure TNF-α levels by ELISA

Result Imiquimod and BI-087 alone without LPS treatment induced IFN-α, TNF-α and IL-1β, while BI-345 did not induce them in human PBMCs. On the other hand, imiquimod and BI-087 also enhanced LPS-induced IL-1β production and BI-087 enhanced LPS-induced TNF-α productions in human PBMCs, however BI-345 suppressed LPS-induced TNF-α and IL-1β production. In mice oral imiquimod and BI-087 induced IFN-α, while BI-345 did not. Only BI-345 suppressed LPS-induced TNF-α among the imidazoquinoline derivatives.

Conclusion Counter screening for LPS-induced cytokine suppression guided us to the BI-345, a cytokine suppressor A small molecular weight orally active synthetic cytokine inhibitor like BI-345 could provide significant beneficial effect in refractory diseases in that TNF-α and/or IL-1β play a major role (*e.g.* autoimmune disease, diabetes mellitus and post-ischemic tissue damage).

Cytokine/ chemokines in allergy

03006

GENETIC DEFECTS IN THE INTERLEUKIN-12 AND INTERFERON-GAMMA PATHWAY

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Immunity to intracellular bacteria such as mycobacteria and Salmonella depends on an effective cell-mediated immune response. The major effector mechanism of this immune response is the activation of macrophages to enhanced microbicidal activity, and is mediated by type-1 cytokines in particular interferon-gamma (IFN-γ). IFN-γ is produced by natural killer cells and T helper-1 cells, and its production is regulated by interleukin-12 and interleukin-18 released by the infected macrophages. Studies on patients with severe, recurrent or atypical infections due to poorly pathogenic mycobacteria and Salmonella have revealed genetic defects in the interleukin-12 and IFN-γ pathway leading to macrophage activation. Many of the patients are either unable to produce or respond to interferon-gamma. This inability results from inherited, genetic mutations in either one of various genes in the type-1 cytokine cascade, e.g. those encoding IL-12Rβ1, IL-12p40 or IFN-γR1. The mutations identified include point mutations and frameshift deletions that lead to recessive-null or dominant-negative phenotypes. The immunological phenotypes resulting from the complete or partial deficiencies in type-1 cytokine (receptor-) genes will be discussed in the context of disease susceptibility and histopathological and clinical manifestations.

03001

CYTOKINE-INDUCED GRANULOCYTE PRIMING IN VIVO IN PATIENTS WITH ALLERGIC ASTHMA.

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Purpose: Chronic inflammatory diseases are characterized by localized inflammatory responses evoked by mechanisms other than clear infections. The tissue damage seen in chronic inflammatory diseases is mediated by activation of extravasated effector cells such as neutrophils, monocytes and/or eosinophils. Extravasation and activation in the tissues is tightly regulated by cytokines and chemokines. To treat this class of diseases an interesting option is to antagonize the pro-inflammatory effector cells specifically, thereby, preventing tissue damage and complaints. Methods: Upon interaction with pro-inflammatory cytokines/chemokines the rapidly change their phenotype and become cells prone for activation. This priming response is important for both adhesion and cytotoxicity associated responses. We have developed antibodies from a semisynthetic phage antibody library to monitor the kinetics of this priming reaction in the peripheral blood of patients with several inflammatory diseases. In addition, we measured the phosphorylation/activation of various signaling molecules on western blots utilizing phosphospecific antibodies. Results: Eosinophils, neutrophils and monocytes are primed in vivo in patients with various inflammatory lung diseases. The degree of priming corresponds with the severity of the disease.
Conclusion: 1. Cytokines released in the lung communicate with inflammatory cells in the peripheral blood. 2. Antibodies directed against priming epitopes on various inflammatory cells provide a new tool to objectively monitor chronic inflammatory pulmonary diseases, such as allergic asthma.

03004

IL-18 INDUCES IgE PRODUCTION IN WILD TYPE AND CASPASE-1 TRANSGENIC MICE: DEPENDENCE ON CD4+ T CELLS, IL-4 AND STAT6.

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Over production of IgE and Th2 cytokines, including IL-4, IL-5 and IL-13, can result in allergic disorders. Although IL-4 is critical to the polarization of naive CD4+ T cells to a Th2 phenotype *in vitro* and in many *in vivo* systems, the factors other than IL-4 that regulate *in vivo* IL-4 production and Th2 commitment are poorly understood. IL-18, an IL-1-like cytokine that requires cleavage with caspase-1 to become active, has strong ability to induce IFN-γ production from T cells, B cells and NK cells, especially in combination with IL-12. Somewhat paradoxically, IL-18 has capacity to induce IL-4 and/or IL-13 secretion from NK cells, T cells, basophils and mast cells *in vitro*. Furthermore, although IL-18 plus IL-12 suppresses IgE production in helminth-infected mice, injection of IL-18 alone into such mice actually increases IgE levels and enhances IL-4 and IL-13 production by their basophils, mast cells and CD4+ T cells. These results taken together suggest that IL-18 may induce IL-4 producing CD4+ T cells or condition cells to make such IL-4 in response to antigenic stimulation. Here we demonstrate IL-18 causes high-level IgE production when administered to normal mice. IL-18-induced IgE production depends upon the action of CD4+ T cells and requires IL-4. Furthermore, skin-specific caspase-1 transgenic mice, with elevated levels of IL-18 in their sera, displayed high serum level of IgE, which is entirely dependent on Stat6. Finally, we show that IL-18 stimulates naive CD4+ T cells cultured in anti-CD3/anti-CD28 coated dishes to develop into cells that produce IL-4 in response to *in vitro* TCR engagement. These results indicate that caspase-1/IL-18 may be critically involved in regulation of IgE production *in vivo*, providing a potential therapeutic target for allergic disorders.

03005

EFFECT OF DER P 1 ON DENDRITIC CELLS DERIVED FROM PATIENTS SENSITIVE TO HOUSE DUST MITE : RELATIONSHIP BETWEEN CD86 AND CD83 EXPRESSION AND TH2 PROFILE.

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The CD80/CD86 costimulatory molecules expressed by antigen-presenting cells interact with CD28/CTLA4 on T cells and might control the polarization of the immune response towards a Th2 or a Th1 profile.
In this work, we evaluated the effect of the cysteine-protease Der p 1, the major allergen of the house dust mite Dermatophagoides pteronyssinus (Dpt) on CD80/CD86 expression by dendritic cells (DC) derived from monocytes purified from subjects sensitive or not to Dpt. Results showed that Der p 1 induced an increase in CD86 expression only on Dpt.allergic patient-derived DC. Simultaneously CD83 was overexpressed. This specific effect on DC was associated with a rapid increase in the production of both proinflammatory cytokines (TNFα and IL-1β and Th2 cytokines (IL-6,IL-10). No IL-12p70 release was observed. These effects were abolished in the presence of the inactive precursor of Der p 1 (ProDer p 1), heated Der p 1, a cysteine protease inhibitor (E64) or in the presence of neutralizing anti-CD23 Fabs. In contrast, similar effects were induced when dendritic cells from only allergic patients were incubated with soluble CD23. Finally purified T cells from allergic patients stimulated by autologous Derp 1-pulsed DC preferentially produced IL-4 rather than IFN-γ. Taken together, these data suggest that by selectively increasing CD86 costimulatory molecule in response to Der p 1, dendritic cells from allergic patients might favour the development of a Th2 response

03003

Interleukin-9 contributes to allergic inflammatory disease by inducing mucus production in the airways.

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Interleukin-9 (IL-9) is a member of the Th2 cytokine family and is suspected to be a major mediator of cellular and physiological responses in asthma. Recently, IL-9 has received considerable attention, due to studies demonstrating genetic linkage of IL-9 and IL-9R gene loci to indices of airway hyperresponsiveness and asthma. Mucus hypersecretion is a clinical feature of chronic airway diseases. However, the mechanisms governing this process are poorly understood. *In vivo* constituive IL-9 production results in a baseline increase of mucus production in the airways. Molecular analyses of lungs from these mice suggest that the increased mucus production is associated with elevated MUC2 and MUC5AC gene expression. Similar results were observed in C57BL/6 mice after intratracheal instillation of recombinant IL-9 but not of IFNγ. *In vitro* human primary lung cultures and a human muccoepidermoid cell line showed similar increase in MUC2 and MUC5AC expression after IL-9 stimulation. In addition, anti-IL-9 antibody treatment inhibits airway hyperresponsivenss and mucin production to natural antigen in a murine model of asthma.
These data support the role for IL-9 in the induction of mucin expression in human and murine epithelia. Our results further extend the functions of IL-9 on regulating cellular responses related to the pathogenesis of allergic inflammatory diseases, including asthma.

03002

MODULATION OF DENDRITIC CELL FUNCTION BY ACTIVATED KERATINOCYTES

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Epidermal epithelial cells, keratinocytes (KC), represent the major constituent of the skin and produce various soluble mediators, such as IL-1β, TNF-α, GM-CSF and prostaglandins, which may have a modulatory activity on the skin immune system. Immature dendritic cells (iDC) reside in the epithelia in close contact with KC. Upon activation iDC undergo final maturation and migrate toward secondary lymphoid tissues, where they prime naive Th cells. Therefore, we questioned whether the products of activated KC could induce final maturation and/or polarization of iDC. To this aim, KC were pre-incubated for 48h with the T cell cytokine IFN-γ and then KC were stimulated for 48h with J558-CD40L cells. KC also pulsed for 2h with poly I:C and chased for 6h or 46h. KC-supernatants (50%, v/v) were added to iDC in the presence of LPS. Supernatants from CD40L-activated KC alone were able to induce maturation in part of DC. When NS-398 (a specific COX-2 inhibitor) was present during KC activation IL-12p70 produced by DC was higher suggesting that PGE_2 (and/or other prostanoids) produced by CD40L-activated KC may have an inhibitory effect on IL-12p70 production by DC. In contrast, supernatants from pI:C-pulsed KC induced the development of DC1. Our results indicate that activated KC may initiate specific immune responses by direct activation and/or polarization of DC.

03009

USE OF PHAGE DISPLAY ANTIBODIES FOR MONITORING CYTOKINE-INDUCED PRIMING OF GRANULOCYTES IN HUMAN PERIPHERAL BLOOD.

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Our research interest is focused on the understanding of human granulocytes pre-activation (priming) induced by cytokines *in vivo*. This process is extremely important in the control of host defence against pathogenic micro-organisms. However, uncontrolled activation may lead to disease such as adult respiratory stress syndrome and septic shock.Granulocyte activation is induced via a multistep process: preactivation by cytokines and/or chemokines followed by activation by e.g. opsonized targets. Preactivation or priming is a prerequisite for optimal downstream activation. The cytokine-induced preactivation or priming of inflammatory cells occurs in the peripheral blood. Monitoring of this priming response is an important means of determination of the dimension of the inflammatory response in the tissues. For this purpose we set out experiments to produce antibodies that can recognise this priming phenotype. After screening of a semi-synthetic phage antibody library of human scFv fragments, two human phage antibodies named MoPhab A17 and A27, were selected for their ability of recognising epitopes expressed on *in vitro* on GM-CSF and TNF-α primed-granulocytes. Furthermore, these antibodies specifically bind to an epitope present in granulocytes of patients affected by obstructive pulmonary disease (COPD) or allergic asthma compared to healthy donors. These data support the use of the two antibodies as tool to study the priming process *in vitro* and to detect the progression of those widespread inflammatory diseases.

03013

ALCOHOL CONSUMPTION UP-REGULATES MURINE Th2 CYTOKINES AND IgE LEVELS

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Chronic alcohol consumption is responsible for a wide variety of health problems. Increased frequency and severity of infections often accompany alcoholism indicating alcohol-induced alterations in innate and adaptive immunity. Our mouse model parallels chronic human alcoholism by polarizing the immune response toward Th2- and away from Th1-driven adaptive immune responses. Ethanol's effect is most pronounced during the early or cognitive phase of the immune response resulting in a profound down regulation of the Th1 cytokines, IL-12 and IFN-γ, and subsequent impairment of delayed hypersensitivity responses. Conversely, alcohol consumption enhances antigen-specific antibody responses. APC from ethanol-consuming mice are mildly glutathione (GSH) depleted and modulation of Th1-Th2 correlates with APC GSH content. The effect of GSH depleted APC on subsequent immune function is profound causing T cells from non-alcohol consuming mice to mature as Th2. We show that alcohol consumption significantly increases IL-9 mRNA levels. We find that total serum IgE levels in unimmunized, alcohol-consuming BALB/c or C57BL/6 mice are 5-8 times greater than in control mice. Kinetic studies show that the mice must consume the alcohol diet for approximately 1 week before we see a significant increase in IgE. When the mice are returned to a normal, non-alcohol diet, IgE levels return to base line levels after approximately 5 days. The result of this Th1-Th2 shift is that the immune system of an alcohol-affected individual may be deficient in fending off intracellular pathogens and exhibit increased asthmatic symptoms.

03008

CONTENTS OF THE INTERLEUKIN-2 AND INTERLEUKIN-6 IN CHILDREN WITH ATOPIC DERMATITIS (AD)

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Expediency of the cytokine study during allergy diseases consists in the necessity of the immunoregulation assess. Contents of the interleukin-2 and interleukin-6 was studied by the immunoferment method in 150 children (53 boys and 97 girls) with atopic dermatitis at the age from 4 month to 14 years. There were 15 practically healthy children in control groups. The increased level of the interleukin-2 was elicited in children with the local form of AD (256,6±0,21 pg/ml).The maximally manifested disbalance of postgraduate cytokines was noted in children with a heavy current of a dermal process. Levels of the interleukine-6 with the remount of the decease less than 2 years didn't differ from results obtained in the control group (25,6±0,21 pg/ml and 32,3±0,41 pg/ml, relatively), contrariwise, the level of the interleukin-2 was slightly increased (64,3±0,5 pg/ml and 33,4±0,31 pg/ml, relatively, $p<0,05$). During the continuous AD there were recorded maximum values of the interleikin-2 contents (435,4±0,32 pg/ml).
Consequently, in protractedly sickening atopic dermatitis children the formation of the immune process is contributed by defections of immunoregulation expressing in hyperproduction of the interleukine-2 and insufficiency of the interleukin-6 synthesis.

03010

EVALUATION OF MURINE MODEL OF ASTHMA INDUCED BY EXPOSURE TO HOUSE DUST EXTRACT CONTAINING HIGH LEVELS OF COCKORACH ALLERGEN.

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Asthma represents a serious health problem particularly for inner city children. Recent studies have identified that many asthmatic attacks are triggered by exposure to indoor allergens including cockroach allergens. However, the mediators within the lung dictating the progression of disease have still not been fully defined. This study tested the hypothesis that asthma-like pulmonary injury may be induced by house dust due to the local recruitment of inflammatory cells especially eosinophil. Households (n=10) with asthmatic children were identified, house dust collected, and tested for cockroach allergens. The sample which tested highest for cockroach allergens (Bla g1=65.8U/ml) was used for subsequent studies. BALB/c mice were immunized with serially diluted house dust extract and received two additional pulmonary challenges. Control mice received sterile PBS only. The inflammatory cells in bronchoalveolar lavage (BAL) and peripheral blood, and myloperoxidase activity in the lung were analyzed. Eosinophil counts and myloperoxidase activity were significantly increased by dose-dependent manner by exposure to the house dust. A kinetics study was then performed using an appropriate dilution of the house dust extract with mice sacrificed every 12 hours after second pulmonary challenge. Inflammatory response reached the peak at 48 hours. In peripheral blood, no meaningful changes in the number of lymphocytes and red blood cells while the number of PMN was augmented in all three immunized groups. Thus, these mice were successfully sensitized and manifested asthma-like responses after house-dust extract challenge containing high levels of cockroach allergens. This murine model may be used for studying the mechanisms of indoor house dust-mediated asthma.

03011

HISTAMINE ENHANCES MIP-1α SYNTHESIS IN PERIPHERAL BLOOD MONONUCLEAR CELLS VIA H_2 RECEPTORS

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Recruitment and activation of basophils is a feature of chronic allergic reactions. The CC chemokine MIP-1α is produced at sites of chronic allergic reactions. Since MIP-1α activates basophils to release histamine, we investigated whether histamine regulates MIP-1α synthesis. Human PBMC were stimulated with LPS, TNF-α or IL-1β in the presence of histamine. MIP-1α synthesis was assessed using a commercially available ELISA. Histamine alone (10 μM) did not induce MIP-1α synthesis. Furthermore, histamine did not affect IL-1α synthesis induced by LPS (0.1 to 10 ng/ml). In contrast, histamine enhanced MIP-1α synthesis induced by increasing concentrations of IL-1α (4.4-fold at 0.1 ng/ml; 3.5 fold at 1 ng/ml; 2.6 fold at 10 ng/ml). IL-1α (10 ng/ml)-induced MIP-1α synthesis was increased 1.5 fold by histamine at a concentration as low as 0.1 μM. The maximal increase in MIP-1α synthesis was seen at 10 μM histamine. Ranitidine, a H_2 receptor antagonist, prevented in a dose-dependent fashion the histamine (10 μM)-mediated increase in IL-1α-induced MIP-1α synthesis. Diphenhydramine, a H_1 receptor antagonist, had no effect. The histamine-mediated increase in MIP-1α synthesis was not limited to IL-1 α since histamine (10 μM) enhanced MIP-1α synthesis induced by increasing concentrations of TNF-α (1.6 fold at 0.1 ng/ml; 2.3 fold at 1 ng/ml; 2.7 fold at 10 ng/ml). Histamine failed to enhance the IL-1α-induced synthesis of MCP-1, another CC chemokine with basophil-activating properties. These findings suggest that histamine released from MIP-1α-activated basophils may contribute to chronic allergic reactions by increasing MIP-1α synthesis in TNF-α- and IL-1β-stimulated mononuclear cells.

03012

ROLE OF IL-5 IN THE EOSINOPHILIA INDUCED BY IL-9 IN VIVO.

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Recent studies point to IL-9 as a potential regulator of eosinophils. In vivo IL-9 production has been shown to potentiate allergen-induced lung eosinophilia, and antibodies against IL-9 block *T. muris* induced blood eosinophilia. To further delineate the role of IL-9 in eosinophil responses, we analysed baseline eosinophilia in transgenic mice constitutively expressing IL-9 in all tissues. Increases in eosinophil numbers were specifically found in bone marrow (x7), blood (x25) but not in other tissues except broncho-alveolar lavage (x25) and the peritoneal cavity (x20). Morphological analysis of sorted eosinophils from IL-9 transgenics showed an unusually high proportion of immature cells in the peritoneal cavity, reflecting bone marrow hyperproduction of eosinophils. Intraperitoneal antigen stimulation dramatically increased eosinophil numbers indicating that IL-9 synergizes with endogenous inflammatory mediators. In this respect, IL-5 was found to be required for IL-9 activity on eosinophils because administration of exogenous IL-9 or expression of the IL-9 transgene failed to induce eosinophilia in an IL-5-deficient background. In vitro, IL-9 did not activate STAT transcription factors in mouse eosinophils and failed to promote eosinophil survival or proliferation even in synergy with IL-5, supporting the hypothesis that its in vivo activity is mediated by an indirect mechanism.

03007

Soluble IL-15Rα suppresses antibody production and prevents allergic inflammation in mice.

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We have recently shown that IL-15 boosts antibody production up to 50-fold in a murine model of allergic sensitization to ovalbumine (OVA) (*Eur. J. Immunol.* 1998. 28:3312). Here we present the first evidence that blocking of endogenous IL-15 by systemic treatment with a soluble IL-15Rα suppresses allergic inflammation and antibody production in Balb/c mice. Sensitization of Balb/c mice to OVA induces antigen-specific antibody production, and intranasal challenge with the antigen initiates a pulmonary allergic inflammation. reatment with soluble IL- 15R α significantly suppresses production of OVA-specific IgE, IgG1, IgG2a and IgM. In addition, blocking of endogenous IL-15 activity by the soluble high affinity receptor prevents induction of a pulmonary allergic inflammation, namely infiltration of eosinophils, neutrophils and lymphocytes as determined by analysis of bronchoalveolar lavage and histological examination of the lungs. The pro-allergic cytokines IL-4 and IL-5 in BAL fluid were highly significantly suppressed by sIL- 15Rα. These data suggest that blocking IL-15 activity by the soluble high-affinity IL-15Rα chain in mice suffices to prevent a model allergic inflammatory response *in vivo*.

Cytokine and interferon gene regulation I

04005

ICSBP (IRF-8) is an essential activator of IL-12p40 transcription and regulates gene expression in macrophages.

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IL-12 is a master cytokine that is required for IFNγ production and T cell mediated immune responses. Of the two subunits, p40 is the inducible subunit that controls overall production of IL-12 in macrophages. IL-12 p40 is transcribed through a pathway activated by IFNγ/LPS, which is separate from the pathway stimulating other cytokines. ICSBP is an IFNγ/LPS inducible transcription factor expressed selectively in macrophages. We have previously shown that ICSBP-/- macrophages are defective in IL-12p40 transcription, which is the principal cause of increased susceptibility to infections in ICSBP-/- mice. In accordance, IL-12p40 promoter activity is stimulated by IFNγ/LPS in ICSBP+/+ macrophages, but not in -/- counterparts, although -/- macrophages were fully capable of inducing IL-1α/β and TNFα. We have shown that transfection of ICSBP into +/+ and -/- macrophages led to activation of both human and mouse IL-12p40 promoters, and that this activation did not require IFNγ/LPS stimulation (J. Immunol in press). Importantly, ICSBP transfection also resulted in the expression of the endogenous IL-12p40 mRNA even in the absence of IFNγ/LPS stimulation. IRF-1, previously implicated for IL-12 expression did not have the capacity to stimulate p40 promoter activity. However, co-transfection of ICSBP and IRF-1 cooperatively enhanced p40 promoter activity. These results show that ICSBP is an obligatory activator of IL-12p40 transcription in macrophages. ICSBP stimulated human IL-12p40 promoter activity through the ETS element previously shown to be critical for activation by IFNγ/LPS. However, ICSBP appears to target many other regulatory elements. For example, it can stimulate transcription through the GAS elements, the classic target for STAT1, thus enhancing nitric oxide and Fc receptor γ transcription in macrophages (PNAS, 97:91, 2000). Our data indicate that ICSBP controls IFNγ/LPS dependent transcription by interacting with other transcription factors. Isolation of a protein that forms a complex with ICSBP upon IFNγ stimulation in macrophages will be described.

04002

Transcriptional Regulation of Chemokine and Interferon Gene Expression by NF-κB and IRF Factors

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Virus infection of host cells activates a set of cellular genes including cytokines, interferons and chemokines, involved in antiviral defense and immune activation. The interferon regulatory factors (IRF) - IRF-3 and IRF-7 - are key activators of the Type 1 interferon genes, as well as the RANTES chemokine gene. Using co-expression analysis, human IFNB, IFNA1 and RANTES promoters were stimulated by IRF-3, whereas IFNA4, A7 and A14 promoters were preferentially induced by IRF-7 only. Chimeric proteins that combined different IRF-7 and IRF-3 domains were also tested and provided evidence of distinct DNA binding properties of IRF-3 and IRF-7, as well as preferential association of IRF-3 with the CBP co-activator. Interestingly, some of these fusion proteins led to supraphysiological levels of IFNA promoter activation. DNA binding site selection studies demonstrated that IRF-3 and IRF-7 bound to the 5'-GAAANNGAAANN-3' consensus motif found in many virus inducible genes; however a single nucleotide substitution in either of the GAAA half-site motifs eliminated IRF-3 binding and transactivation activity, but did not affect IRF-7 interaction or transactivation. These studies demonstrate that IRF-3 possesses a restricted DNA binding site specificity and interacts with CBP, whereas IRF-7 has a broader DNA binding specificity that contributes to its capacity to stimulate delayed type IFN gene expression. Other studies demonstrate that distinct signaling pathways induce IRF-3 phosphorylation, including virus induced C-terminal phosphorylation and stress-induced N-terminal phosphorylation. We further characterized the regulatory control of RANTES transcription during virus infection using *in vivo* genomic footprinting analyses. IRF-3, IRF-7 and NF-κB are identified as important *in vivo* binding factors required for the cooperative induction of RANTES transcription after virus infection. To further characterize the involvement of NF-κB in RANTES induction, retroviral-mediated transfer was used to introduce a dominant form of IKKγ/NEMO subunit (ΔC-IKKγ) into U937 cells. ΔC-IKKγ expression inhibits LPS-, TNF- and virus-induced IKK activity and NF-κB binding activity as well as decreased RANTES gene expression. Strategies to use highly active forms of IRFs as immunomodulatory agents in conjunction with DNA vaccination strategies or as pro-apoptotic agents are also being investigated.

04007

Inducible Expression of IκBα repressor Mutants Inhibits Expression of Cytokine and Apoptotic genes in Jurkat T cells.

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The NF-κB family of transcription factors is involved in the transduction of immunological responses, cellular differentiation and cell growth. Gene knock out and other studies firmly establish a role for NF-κB in immunity and the inflammatory processes, as well as in other functions, such as apoptosis and liver development. Using Jurkat cells engineered to inducibly express a transdominant form of IκBα (TD-IκBα), we examined the role of NF-κB in the regulation of cytokine gene expression at the mRNA level by RNase protection analysis. In this T cell model, expression of TNFα, TNFβ, Ltβ, TGFβ1, TGFβ2 and TGFβ3, as well as IFNγ and interleukin 2 are inhibited following activation of TD-IκBα. We sought to monitor changes in cytokine expression following *de novo* HIV-1 infection and found that TD-IκBα moderately decreased the expression of identified TNFα, TNFβ and Ltβ of following *de novo* infection of T cells with HIV-1. In addition we have found three novel genes regulated NF-κB transcription factor, interleukin 13, I-309 chemokine and TRAIL apoptotic signaling protein. Further investigation revealed a dramatic and rapid increase of intracellular and cell surface TRAIL protein following treatment with a variety of NF-κB inducers. Expression of TD-IκBα resulted in an almost complete downregulation of TRAIL protein, suggesting a possible role for NF-κB in the regulation of TRAIL gene. We have also cloned and tested the TRAIL gene promoter; preliminary experiments using luciferase reporter assays indicate a modest but reproducible activation following activation of NF-κB, as well as a TD-IκBα mediated downregulation of the TRAIL promoter.

04003

IRF Proteins and PU.1 Synergize to Mediate Transcriptional Activation of the Human Interleukin 1β Gene via and Upstream Enhancer Element

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We previously showed that macrophages express Interferon Regulatory Factor (IRF) -4, and that IRF-4 can function synergistically with the Ets-like protein PU.1 to activate human interleukin 1β (IL-1β) reporter gene expression (*J. Immunol.* 163: 2713-2722, 1999). We have now discovered that this synergy occurs through an upstream enhancer element previously shown to confer cytokine- and LPS-inducible expression. The upstream IL-1β enhancer contains a composite PU.1/IRF element that is highly similar to those previously identified in the $gp91^{phox}$, IL-18, and TLR4 promoters. We report here that synergy between PU.1 and IRF-4 requires both DNA contact and protein-protein interaction. Unlike IRF-4, over-expression of Interferon Consensus Sequence Binding Protein (ICSBP) was not capable of significantly augmenting IL-1β reporter activity in macrophages. We found that co-expression of IRF-2 could rescue the ability of ICSBP to activate the IL-1β reporter gene in macrophages. Furthermore, co-expression of PU.1, IRF-1, IRF-2, and either IRF-4 or ICSBP, in fibroblasts resulted in a 10-fold increase in IL-1β reporter activity over that induced by PU.1 and either IRF-4 or ICSBP alone. Similar synergy was observed using a reporter plasmid containing multiple copies of the IL-1β enhancer element. We subsequently co-expressed these transcription factors in human 293 cells, and then assessed their capacity to induce endogenous IL-1β gene expression. Ectopic expression of PU.1 alone was sufficient to activate modest levels of endogenous gene expression, and this expression was markedly increased by co-expression of additional IRF proteins. Together, these data demonstrate that interaction between multiple IRF proteins and PU.1 can act at a single *cis* element to regulate IL-1β expression.

04006

Multiple gatekeepers regulate IRF activity in the early inflammatory response.

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Recent advances have revealed critical role of IRF-3 and IRF-7 factors in innate immunity.We have focused on the identification of the key connections between the activation of IRF-3 and IRF-7 in stimulation of IFNA genes expression and have shown that ectopic IRF-7 can reconstitute IFNA expression in nonproducing cells Although phophorylation of IRF-3 was shown to be needed for the nuclear localization ,we found that receptor mediated phosphorylation was not sufficient.To determine the role of IRF-3 in the induction of IFNA in infected cells we have used IRF-3 directed ribozyme (pU1/IRF3)and have found a correlation between decrease of IRF-3 levels and induction of the endogenous IFNAs in infected cells.Also expression of IRF-7 DN mutant or HHV-8 encoded vIRFs which form nonfunctional heterodimers both with IRF-3 and IRF-7 suppressed IFNA expression. To determine the factors that regulate expression of IRF-7 gene we have isolated IRF-7 promoter and identified the ISRE like element that confers sensitivity to Type I IFNs. The element and mechanism responsible for silencing of IRF-7 expression in some cells lines were identified. Finally we have cloned a novel, previously uncharacterized IRF-5 and functionally characterized this protein. Altogether these studies offer a new approach to the treatment of inflammatory disease by modulation of a specific transcription control.

04001

ROLE OF IRF-1 IN THE REGULATION OF IL-18 PRODUCTION, RELEASE AND BIOACTIVITY

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IRF-1 is a transcription factor involved in the regulation of several cytokines. Using IRF-1 KO mice, the role of IRF-1 in the regulation of IL-18 production, release and bioactivity was investigated. No significant differences in constitutive IL-18 mRNA and tissue-associated IL-18 protein levels were observed between wild type (WT) and IRF-1 KO mice. Administration of IL-12 (400 ng/mouse/day for 4 days, i.p.) or ConA (200 μg/mouse, i.v.) had only minor effects on IL-18 mRNA and tissue-associated IL-18 protein in both WT and IRF-1 KO mice. However, unlike WT mice, administration of either IL-12 or ConA did not increase serum IL-18 levels in IRF-1 KO mice, suggesting a deficient IL-18 release in the absence of IRF-1. Accordingly, both constitutive and inducible caspase-1 mRNA levels were strongly and significantly reduced in the liver and the spleen of IRF-1 KO compared to WT mice, suggesting that the lack of circulating IL-18 in IRF-1 KO mice is due to deficient caspase-1 expression. Absence of IRF-1 also led to a marked reduction of constitutive and inducible mRNA expression of the naturally occurring IL-18 antagonist, IL-18 binding protein (IL-18BP). This latter finding is likely secondary to reduced IFNγ levels in IRF-1 KO mice. IRF-1 therefore regulates IL-18 activity at two separate and contrasting levels: lack of IRF-1 is associated with reduced release of IL-18 from the intracellular compartment, but also with reduced expression of IL-18BP. IL-12 and other IFNγ-inducing cytokines are known upregulators of IRF-1 expression. Compared to IL-12, IL-18 had only a minor effect on IRF-1 mRNA expression in cultured murine splenocytes. Furthermore, induction of IFNγ by IL-18 was only partly dependent upon IRF-1.

04022

IL-4/STAT6 REPRESSES STAT1 AND NF-κB DEPENDENT TRANSCRIPTION THROUGH DISTINCT MECHANISM

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STAT6 mediates IL-4-dependent positive and negative regulation of inflammatory gene expression. In the present report we examined the molecular mechanisms involved in IL-4-induced repression of reporter gene transcription driven by STAT1 and/or NF-κB. Transient expression of STAT6 in a STAT6-deficient cell line (HEK 293) conferred sensitivity to IL-4 for STAT6-dependent transcription and for repression of IFNγ/STAT1- and/or TNFα/NF-κB-driven reporter gene expression. In cells transfected with STAT6 lacking its transactivating domain, IL-4 could not mediate either positive or negative control of reporter gene expression. Over-expression of CBP enhanced IL-4/STAT6-stimulated transcription and overcame IL-4-mediated repression of TNFα/NF-κB-dependent but not IFNγ/STAT1-dependent transcription. A single amino acid change in the DNA binding domain of STAT6 (H415A) eliminated both transcription from the IL-4 responsive promoter and suppression of TNFα/NF-κB-stimulated reporter gene transcription. Nevertheless this DNA binding mutant retained nearly full capacity to suppress IFNγ/STAT1 mediated transcription. Taken together these results demonstrate that STAT6-mediated suppression of STAT1 or NF-κB mediated transcription involves distinct mechanisms. Both processes are dependent upon the STAT6 TAD and appear to involve sequestration of different transcriptional co-activator proteins which may depend upon differential DNA binding interactions.

04021

IRF-2 REGULATES CELL GROWTH AND DIFFERENTIATION VIA INTERACTION WITH HISTONE ACETYLASES.

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We have previously shown that IRF-2-dependent transcriptional regulation was mediated by PCAF (ISICR 1999 in Paris). In this report, we show that p300 also mediates IRF-2-dependent transcription and IRF-2 is acetylated by these histone acetylases. Recombinant IRF-2(rIRF-2) is acetylated in vitro by rp300 and rPCAF. IRF-2 was associated with p300 much less than with PCAF, however, p300 acetylated IRF-2 more efficiently. IRF-2 DNA binding activity was not changed despite of its acetylation in a gel shift analysis. IRF-2 was acetylated after association with PCAF and p300 in TPA-treated U937 cells. In addition, adenovirus EIA12S cotransfection inhibited IRF-2 increased H4 promoter in NIH3T3 cells, suggesting that endogeneous p300 mediates IRF-2-dependent H4 gene activation. These data indicate that IRF-2 acetylation is involved in cell growth regulation, discussing a novel mechanism in which acetylation by histone acetylase regulates IRF-2 activity.

04023

VIRUS-INDUCED TNF-αEXPRESSION IN MACROPAHGES IS REGULATED BY A DUAL MECHANISM: TRANSCRIPTIONAL REGULATION BY NF-κB AND ATF/JUN AND TRANSLATIONAL REGULATION

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Here we have investigated the regulation of TNF-α expression in macrophages during herpes simplex virus (HSV) infection. Despite a low basal level of TNF-α mRNA present in resting macrophages no TNF-α protein is detected. HSV infection marginally increases the level of TNF-α mRNA and protein in resting macrophages whereas a strong increase is observed in IFN-γ-cotreated cells. We found that production of TNF-α was regulated at the transcriptional level. Moreover, we observed that treatment of the cells with actinomycin D, which totally blocked mRNA synthesis, only partially prevented accumulation of TNF-α protein, indicating that the infection lifts a block on translation of TNF-α mRNA. By EMSA we found that binding to the κB#3 site was induced within 1 h after infection and persisted beyond 5 h where TNF-α expression is down-modulated. Binding to the CRE site was also induced but more transiently with a kinetics closely following activation of the TNF-α promoter. Supershift analysis revealed that p65 and p50 were the main κB-binding components while ATF2 and Jun occupied the CRE site. No inducible binding was observed to the binding sites for AP-1, NF-AT and Sp1. Specific inhibitors against either NF-κB activation or the ATF2 kinase p38 abrogated TNF-α expression, showing a requirement for both signals for activation of the promoter. Reporter gene assays confirmed that HSV-induced TNF-α expression in macrophages requires both NF-κB and ATF2/Jun. As to the translational regulation of TNF- we are exploring the potential involvement of the AU rich region localized in the 5' UTR – a region reported to regulate TNF- mRNA translation in response to e.g. lipopolysaccharide.

04019

EMBRYONIC INTERFERON-GAMMA GENE EXPRESSION IS CORRELATED WITH TIGHT JUNCTIONS SEALING IN PIG TROPHOBLAST CELLS

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A strong expression of interferon-gamma (IFN-γ) by trophectoderm of the pig embryo at the time of attachment to the maternal endometrium (day 12 to 18) was described few years ago. This atypical, transient and developmentally regulated expression raises interesting questions about the mechanisms of IFN-γ gene induction in this tissue. In order to study these mechanisms, a cloned cell line from peri-implantatory pig trophectoderm was isolated. When these cells are grown on microporous membrane they differentiate into highly polarized cell monolayer in which tight junctions (TJs) formation, as monitored by transepithelial resistance (TER) increase, is accompanied by a transient expression of IFN-γ in the apical compartment of the cell culture system within 4-6 days. Moreover, when these polarized cells are stimulated for 1 hour to 10 days with phorbol esters (PMA), a dramatic decrease of the TER and a large increase of permeability to Dextran-FITC and horseradish peroxidase was observed indicating TJs slackening. After PMA removal, TER raises to prestimulation values and this increase also correlates with a transient IFN-γ mRNA and protein expression which is 5 to 10 fold higher than the spontaneous one. This is the first example of inducible expression of IFN-γ by a polarized epithelial cell. Moreover, our results suggest that IFN-γ gene induction is regulated by cell contact signaling in this system. Indeed, several studies have shown that junctions between adjacent cells could influence many aspects of epithelial cell physiology. The effects of other factors known to transiently affect TJs scaling on IFN-γ gene induction are under study as well as the intracellular transducing proteins involved in this phenomenon (e.g. G proteins, protein kinase C and tyrosine kinases).

04017

PRODUCTION OF IFN-γ BY MOUSE ERYTHROID NUCLEAR CELLS.

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Previous research showed the ability of mouse erythroid nuclear cells to suppress the immune humoral response in vitro and by adaptive transfer in vivo. It was demonstrated that the protein synthesis is needed for this effect of suppression, and the effect is mediated by the soluble factor. We have studied IFN-γ gene expression and IFN-γ production by erythroid nuclear cells, which can mediate the immunosuppression. Mouse erythroid nuclear cells isolated from spleen with erythroid hyperplasia (after acute hypoxia or phenilhydrazine-induced anemia) expressed IFN-γ mRNA. The amount of IFN-γ mRNA expressed by erythroid nuclear cells were comparable with amount of IFN-γ mRNA expressed by splenocytes of immunised mice. Further we have studied IFN-γ production by erythroid nuclear cells isolated from spleen with erythroid hyperplasia and cultivated 24 hour without Epo. Conditional media of erythroid cells ($1\text{-}5x10^6$ cell/ml) was obtained and IFN-γ concentration measured by method of the electrochemiluminescence using poly- and monoclonal antibodies. Measured IFN-γ production of erythroid nuclear cells was 21011,3 pg/ml after acute hypoxia, 6475,02 pg/ml after phenylhydrazine-induced anemia and after bleeding 4868,34 pg/ml which is comparable with production of thymic cells and splenocytes after mitogenic stimulation. We conclude that immunosuppressive effects of erythroid cells on humoral immunity could be mediated by IFN-γ production.

04016

FUNCTIONAL RECONSTITUTION AND REGULATION OF IL-18 ACTIVITY BY IL-18Rβ CHAIN

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The unique synergism between IL-18 and IL-12 in IFN-γ production remains unclear. We examined the role of the IL-18 receptor β chain (IL-18Rβ) in human PBMC, the human NKO cell line and COS-1 cells. PBMC and NKO cells constitutively express both IL-18R α and β chains and produce IFN-γ in response to IL-18 plus IL-12. Although COS-1 cells produce IL-8 when stimulated by IL-1β or TNFα, they do not respond to IL-18, despite abundant expression of the IL-18Rα chain. COS-1 lacks the IL-18Rβ chain. The human IL-18Rβ cDNA was cloned and COS-1 cells were transiently transfected with the IL-18Rβ chain and a luciferase reporter. In transfected cells, IL-18 induced alone luciferase and IL-8, independent of IL-1, TNF or IL-12. Nevertheless, monoclonal antibody to the IL-18Rα chain prevented IL-18 responsiveness in COS-1 cells transfected with the IL-18Rβ chain. In the human NKO cell line, IL-18Rα and IL-18Rβ are expressed constitutively but IL-18 did not induce the synthesis of IFN-γ unless IL-12 was present. IL-12 increased steady state levels of both IL-18Rα and IL-18Rβ mRNA and the production of IFN-γ corresponded to this IL-12-induced effect. Even at high concentrations of IL-12, IFN-γ production was not affected by the IL-18 binding protein. These studies demonstrate that the IL-18Rβ chain is essential for IL-18 activity and support the concept that IL-12 regulates the expression of both chains of the IL-18 receptor complex.

04015

UNRAVELING THE ROLE OF PROTEIN-PROTEIN INTERACTIONS IN IRFS ACTIVITY: A DOMINANT NEGATIVE APPROACH

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Protein-protein interactions play crucial roles in the activity of Interferon Regulatory Factors (IRFs) and different protein complexes have been reported in various immune cells. Previously, we showed that the DNA Binding Domain (DBD) of ICSBP is an independent module that is essential for the formation of DNA binding heterocomplexes with other IRF and non-IRF transcription factors. In addition, we identified the association domain of ICSBP that is essential and sufficient for its interaction with IRF1 and IRF2. This region, termed IRF Association Domain (IAD), shows significant homology in all IRF members excluding IRF1 and IRF2. The IAD also mediates interaction and transcriptional synergy of ICSBP with non-IRF members such as the lymphoid essential factors PU.1 and E47. These interactions were first described for IRF4, which demonstrates the highest homology to ICSBP and is also lymphoid specific. These data suggested that ICSBP defective in its DBD would still be able to interact with other transcription factors but the heterocomplex would not be able to bind to Interferon Stimulated Response Elements (ISRE) or to IRF composite elements. Therefore, ICSBP defective in its DBD could serve as a dominant negative (DN) factor that can compete with endogenous ICSBP for the interactions with IRF and non-IRF transcription factors. We found that DN-ICSBP can compete in transient transfection assays for interaction of wild type ICSBP with IRF1, IRF2, PU.1 and E47, thus negating the activity of these complexes on a given promoter. In addition, DN-ICSBP could also compete with IRF4 for interactions with PU.1 and E47. In accordance, IRF4 defective in its DBD could also compete with wild type IRF4 and ICSBP for interactions with PU.1 and E47 in transient transfection assays. In order to study protein-protein interactions crucial for IRF activity *in-vivo*, the use of a bicistronic retroviral vector for expression of DN-ICSBP in hematopoietic cell lines was employed. The effect of DN-ICSBP on expression of IRF regulated genes, such as MHC class I and immunoglobulin light chain will be discussed. Our approach allows to study the various IRF interactions *in-vivo* and their effects on various aspects of the immune system.

04014

INHIBITION OF 2'-5' OLIGOADENYLATE SYNTHETASE BY ZINC.

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The 2'-5' Oligoadenylate synthetase (OAS) constitute a unique family of interferon induced enzymes, which catalyses the formation of 2'-5' linked oligoadenosine (2-5A). 2-5A binds to and activates a latent endo-ribonuclease (RNase L) leading to degradation of cellular as well as viral RNA. This pathway is a part of the interferon induced antiviral defence present within all mammalian cells. Recent research has proposed a role for OAS in the induction of apoptosis in human cells. Since zinc can protect against apoptosis we have investigated the effect of zinc on OAS activity. Our result shows that zinc is a potent inhibitor of OAS activity, with an IC_{50} value of 99 μM for the p42 isoform and 20 μM for the p69 isoform. Furthermore we have studied the effect of zinc on the multimeric state of the p42 isoform and found that zinc induces multimerisation of the p42 isoform of OAS in a concentration dependent manner similar to that seen for inhibition. Therefor we propose that high zinc concentration within a cell lead to the formation of an inactive OAS multimeric complex

04013

COMPARATIVE ANALYSES OF IRF-3, IRF-7 AND IFNA GENE EXPRESSION IN HUMAN TYPE 2 DENDRITIC CELL (DC2) PRECURSORS VS. MONOCYTES AND MONOCYTE DERIVED DC1.

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Human peripheal blood natural IFN producing cells (IPC) are "professional" IPC that respond to enveloped viruses with high levels of IFN-α production. These cells have recently been identified as rare (0.3% of PBMC, but also present in bone marrow, tonsil, spleen and lymph node), CD123+ (IL-3R), Lin-, HLA-DR+ cells identical to precursors of the type 2 dendritic cell (pDC2) and produce 3-10pg of IFN-α/cell in response to HSV. In contrast, monocytes produce 10-fold less IFN/cell, and monocyte derived DC (DC1) produce <.01 pg/cell) in response to HSV. Two transcription factors are known to be involved in the activation of type 1 IFN genes, namely IFN regulatory factor (IRF)-3 and IRF-7. We have compared the relative expression of IFN-α mRNA as well as mRNA from these two IRFs in highly enriched pDC2s (selected by immunomagnetic sorting) and compared these to expression by monocytes and DC1 (obtained by 7 day culture of monocytes with GMCSF and IL-4) upon Sendai virus or HSV stimulation. pDC2 expressed IFNA message at high levels in response to both HSV and Sendai, whereas monocytes responded well to Sendai and weakly to HSV, and DC1 responded weakly to Sendai and not at all to HSV.Our results indicate differential constitutive expression of IRF-7 between the high and low IFN producers, which does not increase upon viral stimulation, while the levels of IRF-3 were similar in each of the cell types. We have also compared the expression of individual IFNA subtypes induced in these cells by using RT/PCR and sequence analyses of the amplified IFN cDNAs. We found that the major IFNA subtype in each of the cells types is IFNA1, however there were differences in the compositions of the other IFNA subtypes between high and low IFN producing dendritic cells as well as differences with HSV vs. Sendai stimulation of the pDC2. Further details of these studies will be presented.

04012

SEQUENTIAL INTERFERON GENE EXPRESSION IN U937 CELLS.

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One possible reason for the genetic complexity of the interferon-α/β (IFN) gene family was recently presented as Levy and collaborators (Marie et al., EMBO J 1998;17:6660-9.) demonstrated that IFN-genes in mice were expressed in a cascade-like manner. In the first phase IFN-α4 and IFN-β were expressed and in a parakrine way induced the expression of other IFN-α genes in a second phase. In a search for a similar pathway in the human system, we employed the promonocytic cell line U937, induced by Sendai virus, as this cell line produces IFN at levels comparable with human peripheral leukocytes. The model builds on the assumption, that the first phase of genes is induced in the absence of protein synthesis, and the second phase requires expression of the IFN-receptor. Our approach was to study IFN-expression in U937 cells in the presence of cycloheximide and in a interferon resistant variant of U937. In order to distinguish between different IFN-genes, amplification of IFN mRNA by RT-PCR using subtype specific primer pairs, nested priming, restriction cleavage and sequencing were employed. In this study we showed that the expression of IFN-α2, IFN-α8 and IFN-β was independent of protein synthesis, while IFN-α1, IFN-α10, IFN-α13, IFN-α14 and IFN-α17 required protein synthesis.

04011

Regulation of the expression of IFI-16, a HIN-200 family protein induced by interferons.

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IFI-16 is a member of the HIN-200 family of proteins that is constitutively expressed in some cell types, but is induced by type I and type II interferons in others. We sought to understand the mechanisms underlying the regulation of IFI-16 expression by cloning and sequencing the 5' promoter of IFI-16 and performing reporter gene assays using wild type and deletion mutant constructs. In both Hela cells, that constitutively express IFI-16, and HL-60 cells, that express the protein in response to interferons, we identified a 25bp sequence that was sufficient and necessary for activity of the promoter. Introduction of this sequence designated IFI-16 activation element (IAE), conferred transcriptional activity on a previously inactive promoter that was lost when the sequence was mutated. The IAE contained consensus sequences for the binding of IRF1/2 and AP-1 proteins and EMSA studies revealed a single major specific binding complex in nuclear extracts from Hela cells and primary human fibroblasts. This binding activity was competed by oligonucleotides containing AP-1 binding sites but not by IRF binding site containing oligonucleotides and formation of the complex was dependent on an intact AP-1 binding site. Finally, the IAE binding complex could be super-shifted by antibodies that recognise Jun family proteins. We concluded that constitutive expression of IFI-16 in Hela cells was mediated through an AP-1 binding site by a complex that includes a Jun family transcription factor. These data suggest that induction of AP-1 DNA binding activity may represent a novel mode of interferon inducible gene expression.

04009

IRF-3 AND IRF-7 DIFFERENTIALLY RECOGNIZE THE DNA MOTIFS PARTICIPATING IN VIRUS-INDUCED TRANSCRIPTION OF THE MURINE IFN-A4 GENE PROMOTER

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The Virus-Responsive Element of the murine IFN-A4 gene promoter (VRE-A4) contains four modules with different enhancing properties : the module A, represented by the [-103 to -93] GTAAAGAAAGT sequence, uninducible even in multiple copies; the B and C modules corresponding respectively to the [-98 to -87] GAAAGTGAAAAG and [-85 to -74] GAATTGGAAAGC sequences are virus-responsive once dimerized or in combination with each other; and the D module represented by the [-57 to 45] GAAAGGAGAAACT sequence cooperates with the minimal inducible element formed by the first three modules, to confer maximal NDV-inducibility to the IFN-A4 promoter in L929 cells. IFN-A4 promoter activation by IRF-3(5D), a constitutively active form of IRF-3, was dramatically reduced by the –78A/G and –57G/C double mutations of IFN-A4, that affect the C and D modules. The decrease observed in the transactivation of the promoter (from 180-fold to 8-fold) was correlated with a 5-fold decrease in the IRF-3 binding affinity as shown by competition experiments performed with IRF-3 DNA-binding domain. Data obtained with the minimal virus-responsive sequences of IFN-A4, indicated that the lack of the C module affected particularly the IRF-3 response, whereas the lack of the D module affected, although moderately (2-3 fold), either the IRF-3- or IRF-7-mediated transcription. We showed that the C module corresponds to a preferential binding site for IRF-3, whereas the distal region of VRE-A4 containing the A and B modules constitutes a preferential binding site for IRF-7. These results also indicated that the lack of any of the A, B or C module considerably altered the promoter response to IRF-3 and IRF-7 and that cooperation among the four modules is required for maximal transactivation by these factors.

04008

CHARACTERIZATION OF INTERFERON REGULATORY FACTOR-5 AND ITS ROLE AS A REGULATOR OF IFN-α/β GENE PROMOTERS

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A family of interferon (IFN) regulatory factors (IRFs) have been shown to play a role in the transcriptional activation of IFN genes as well as IFN-stimulated genes and other cytokines and chemokines. Some of these IRFs are virus-inducible and IFN-inducible thus play a distinct role in antiviral defense, cell growth regulation and immune activation. The IFR-5 sequence had been previously identified as a member of the IRF family based on homology with other members at the DNA-binding domain which resides in the N-terminal region. Human IRF-5 is most homologous to the uncharacterized IRF-6, IRF-7b, ICSBP and IRF-4. In addition, IRF-5 is unique to the other IRFs in that it contains two nuclear localization signals, one N-terminal and the other C-terminal. We have isolated human IRF-5 by a yeast two-hybrid assay from B cell library and as ETS cDNA cloned from dendritic cells. By RT-PCR, we demonstrate that IRF-5 is constitutively expressed at low levels in a variety of cell types. However, by Northern blot analysis, IRF-5 mRNA was primarily detected in tissues and cells of lymphoid origin. Dependent upon the cell lineage, IRF-5 can be induced by infection with SeV, NDV or by treatment with IFN α. Expression of IRF-5 as a Gal4 fusion protein activated expression of the CAT reporter gene to a greater extent than IRF-1, indicating that IRF-5 contains a transactivation domain. Furthermore, overexpression of IRF-5 in infected cells stimulated expression of an ISRE-containing promoter more efficiently than IRF-3 or IRF-7. By transient transfection, Hela cells overexpressing IRF-5 activated IFNA1 and IFNB SAP reporter plasmids as efficiently as IRF-7. Collectively, these data indicate that IRF-5 may play a similar role as IRF-3 and 7 in the activation of IFN-α/β gene promoters. A functional characterization of this new IRF will be presented.

04020

DIMERIZATION IS THE KEY EVENT OF IRF7 ACTIVATION IN RESPONSE TO VIRAL INFECTION

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Interferon Regulatory Factor 7 (IRF7) is an interferon-inducible transcription factor which has been shown by us and others to play an essential role in the induction of IFNα and β genes in response to viral infection. Potent transactivation of IRF7 is masked in the latent protein by the presence of an autoinhibitory domain that is capable of silencing a strong bipartite transactivation domain. Repression is overcome by conformational changes elicited by viral-induced phosphorylation on serine residues that map to the extreme C-terminal region of the protein. Deletion of the autoinhibitory domain leads to potent transactivation independent of virus-induced phosphorylation. Phosphorylation of IRF7 also triggers enhanced dimerization, nuclear retention and increased DNA binding. A conditionnally dimerizable form of IRF7 was constructed by fusing IRF7 with the ligand-binding domain of estrogen receptor (ER). Hormone-dependent dimerization of chimeric IRF7-ER stimulated specific DNA binding and transcriptional activation of endogenous IFNα genes. These results show that phosphorylation-dependent dimerization of IRF7 in virus infected cells is the likely mechanism regulating its function. Finally, additional posttranslational modification events may further modulate IRF7 activity.

04024

IRF7 IS ACTIVATED BY DIMERIZATION FOLLOWING PHOSPHORYLATION BY A NOVEL VIRUS-STIMULATED KINASE SENSITIVE TO INHIBITION BY RNA-BINDING PROTEINS

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Interferon regulatory factor 7 (IRF7) is an IFN-inducible transcription factor required for activation of a subset of IFN-alpha genes that are expressed with delayed kinetics following viral infection. IRF7 is synthesized as a latent protein and is post-translationally modified by protein phosphorylation in virus infected cells. Phosphorylation requires a carboxyl-terminal regulatory domain that controls retention of the active protein exclusively in the nucleus, its binding to specific DNA target sequences, multimerization, and ability to induce target gene expression, Transcriptional activation by IRF7 mapped to two distinct regions, both of which were required for full activity, while all functions were masked in latent IRF7 by an autoinhibitrory domain mapping to an internal region. A conditionally active form of IRF7 was constructed by fusing IRF7 with the ligand-binding and dimerization domain of estrogen receptor (ER). Hormone-dependent dimerization of chimeric IRF7-ER stimulates DNA binding and transcriptional transactivation of endogenous target genes in the absence of virus infection or protein phosphorylation. These results demostrate that protein dimerization is the key regulatory step inducing IRF7 activity and suggest that virus-induced phosphorylation causes allosteric changes that stimulate dimerization, leading to nuclear retention, unmasking of DNA binding, and derepression of transactivation. Yeast two-hybrid and protein-interaction experiments indicate that the IRF7 DNA binding domain is a target for both intra-molecular and inter-molecular interactions in both the silent monomer and activated dimer. Futhermore, interaction studies have identified potential components of the virus-stimulated signaling pathway leading to IRF7 phosphorylation and IFN gene induction.

04025

DIFFERENTIAL CONTRIBUTION OF IL-1 RA ISOFORMS TO ALLELE-SPECIFIC IL-1RA mRNA ACCUMULATION

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Two isoforms of interleukin-1 receptor antagonist (IL-1ra), intracellular (icIL-1ra) and secreted (sIL-1ra), have been described. Synthesis of each isoform is controlled by distinct promoter regions. Five IL-1ra gene alleles exist, and several single base polymorphisms further distinguish two of the most common alleles, 1 and 2. Although differences in IL-1ra protein production have been demonstrated in individuals carrying allele 1 vs. allele 2, underlying mechanisms of such disproportion are poorly understood. We hypothesized that variations in the synthesis of allele-specific IL-1ra mRNA may be responsible for the observed disparity in IL-1ra protein production. Using RT-PCR and restriction digestion analysis, we studied accumulation of icIL-1ra and sIL-1ra allele-specific mRNA in colonic biopsy specimens and PBMC of heterozygous (1/2) individuals (n=8). Our data showed no difference in the amount of sIL-1ra allele-specific mRNA transcripts. For icIL-1ra, however, relative amounts of allele 1 - specific mRNA were four times greater than that of allele 2. A single base difference found in the icIL-1ra promoter may be responsible for the observed effect. Using a 1.2 kb fragment of the icIL-1ra promoter region in transient transfection studies, we found that transcription from the allele 2 - specific promoter was 30% lower than from the allele 1 - specific promoter, thus indicating that the disparity in icIL-1ra allele-specific mRNA accumulation may be due to the difference in activity of the corresponding promoters. Taken together, our data suggest that functional polymorphisms in the icIL-1ra gene may play a role in the onset and/or perpetuation of chronic inflammatory diseases.

04026

PU.1 REGULATES INTERLEUKIN-1 RECEPTOR TYPE II GENE EXPRESSION IN MYELOID CELLS.

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Interleukin-1 receptor type II (IL-1RII), a functional antagonist of IL-1, is primarily expressed on mature myeloid cells. In differentiated myeloid cell lines, the IL-1RII gene is transcribed exclusively at the distal alternate first exon. Although IL-1RII gene is transcribed at both distal and proximal first exons in the EBV-immortalized B cell line RAJI, the lack of IL-1RII gene expression in the EBV-negative B cell line BJAB indicates that IL-1RII gene expression is not a feature of B cells. The present study was designed to identify the cis-regulatory elements and transcription factors required for the myeloid-restricted use of the distal IL-1RII promoter. A 956 bp fragment (-905/+51) increased basal luciferase activity of the promoterless vector pXP2 41-fold in the acute monocytic cell line U937 and 22-fold in RAJI cells, but only 5-fold in BJAB cells. Deletion mutants indicated that a 128 bp fragment (-79/+51) was sufficient to confer maximal promoter activity in U937 cells. A further deletion to create the fragment –40/+51 sharply reduced (-48%) promoter activity. A similar reduction (-49%) was obtained by point mutations in the PU.1 consensus motif –55/-46. In EMSA, a single complex was detected upon addition of nuclear extracts to probe A encompassing the site –55/-46. In competition experiments, point mutations in the site –55/-46 rendered probe A unable to abolish the single complex. A role for PU.1 was suggested in competition experiments with probes carrying either the functional or mutated PU.1 site from the CD11b promoter. Transient expression of PU.1 in the PU.1 negative hepatoma cell line $HepG_2$ was sufficient to reveal the distal IL-1RII promoter activity. In U937 cells, a deletion of the 5'UTR to create the fragment –79/+14 nearly abolished (-80%) promoter activity. Sequence analysis revealed a purine-rich region (+42/+33) with high homology to the PU.1/NFβA site found in the IL-1β promoter. In EMSA, three complexes were generated when nuclear extracts from U937 cells were incubated with probe D (+11/+51) or D3 (+27/+51). All complexes were competed by the wild-type CD11b probe, but not by the CD11b probe carrying a mutated PU.1 site. Further studies will address the nature of the transcription factors cooperating with PU.1 to promote IL-1RII gene expression during myeloid differentiation.

04027

LEFLUNOMIDE INHIBITS IL-6 AND NITRIC OXIDE PRODUCTION IN L929 FIBROBLASTS IN A STIMULUS-SPECIFIC MANNER

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Leflunomide is an immunosuppressive drug which shows beneficial effects in autoimmune diseases and transplantation. Since activated fibroblasts, by producing proinflammatory cytokines and nitric oxide (NO), may contribute to the autoimmune effector mechanisms, as well as graft rejection, the aim of this study was to investigate effects of leflunomide on the production of proinflammatory mediators, IL-6 and NO, in L929 fibrosarcoma cell line. Leflunomide's active metabolite, A77 1726, in a dose dependent manner, inhibited IFN-γ or IFN-γ/LPS-induced NO and IL-6 production. Recently it has been reported that decreased NO level may reduce IL-6 production in murine lung fibroblasts. However, it seems that leflunomide-mediated inhibition of IFN-γ-induced IL-6 synthesis in L929 cells was not a consequence of impaired NO production, since in this cell type well known inhibitors of NO generation, aminoguanidine and L-NAME, did not change IFN-γ-triggered IL-6 production. Interestingly, leflunomide affected neither NO nor IL-6 production induced by cAMP elevating agent, rolipram, indicating that different mechanisms are responsible for rolipram and IFN-γ mediated stimulation of IL-6 and NO production in L929 cells. Together, these findings suggest that leflunomide-mediated inhibition of IFN-γ-triggered IL-6 and NO production in cells of fibroblast origin could contribute to the observed beneficial effects of this drug in transplantation as well as in autoimmunity.

04028

ACTIVATION OF INTERFERON-RESPONSE FACTOR IRF-7 BY VIRAL INFECTION

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Activation of the Interferon Response Factor family member IRF-7 has recently been shown to be important in the induction of the full-range of IFN α production in response to viral infection. This study investigates the mechanisms of IRF-7 regulation following viral infection. IRF-7 is shown to be phosphorylated in response to viral infection and this phosphorylation is critical for IRF-7 nuclear localization and DNA binding. This phosphorylation can be inhibited by staurosporin, a broad specificity serine/threonine kinase inhibitor. However, IRF-7 phosphorylation is not inhibited by inhibition of the IkB kinase, PKR, PKC, PKA, or PI3K. In addition, inhibition of the MEK and p38 kinases failed to affect IRF-7 phosphorylation in response to viral infection. Additional studies into the regulation of IRF-7 phosphorylation have lead to the finding that the vaccinia virus E3L protein can inhibit the phosphorylation of IRF-7 in response to viral infection. E3L is a double stranded RNA binding protein known to inhibit the PKR protein kinase. However, another vaccinia virus protein, K3L, that also inhibits PKR, failed to inhibit IRF-7 phosphorylation. Furthermore, IRF-7 is still phosphorylated in PRK -/- cells and these cells show normal IFN production in response to viral infection. In a parallel line of experiments, E3L was also shown to inhibit IRF-3 phosphorlation in an analogous manner. These results suggest that the E3L protein can inhibit another non-PKR cellular kinase and that this kinase is required for phosphorylation of both IRF-3 and IRF-7 in response to viral infection. Structure/function studies of IRF-7 have shown that C-terminal sequences of the protein are important in both the regulation of DNA binding ability as well as the proteins cellular localization. Phosphopeptide mapping studies have localized IRF-7 phosphorylation to this same region, suggesting that the C-terminus of IRF-7 plays a critical regulatory role.

04029

CHARACTERIZATION OF THE PROXIMAL PROMOTER OF THE HUMAN IL-12 RECEPTOR ß2-GENE AND THE IDENTIFICATION OF PUTATIVE SILENCER ELEMENTS

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A high affinity receptor for IL-12 consists of two subunits, the IL-12Rß1 and IL-12Rß2. The latter is expressed on Th0 and Th1 cells but not on Th2 cells, making these cells unable to respond to IL-12. The mechanisms controlling the expression of the IL-12Rß2 gene are still largely unknown. To start elucidating these mechanisms, we cloned a 1.2 kb fragment immediately to the transcription start site and tested its activity in a reporter gene assay in Jurkat cells. Gene expression driven by this promoter region after TCR stimulation was more than 10-fold greater than by the empty vector. Truncation of the fragment to - 0.6 kb increased promoter activity, suggesting the presence of negative regulatory elements (NREs). Mutation of a GGGCGG motif (SP1/3 binding site) at –64 bp reduced promoter activity, whereas mutation of the TTTCC motif (NFAT binding site) at –209 bp increased activity. The region –220/+49 bp was sufficient for basal and inducible activity. Mutation of GATA motifs in the NREs (at -886 bp and –1033 bp) increased promoter activity. These results suggest that the regulation of inducible transcription of the IL-12Rß2 promoter in T cells involves NFAT (-) and SP1/3 (+). Since GATA3, an IL-4-promoted and Th2-specific transcription factor, down-regulates IL-12Rß2 protein expression, it is tempting to speculate that GATA3 downregulates IL-12Rß2 expression directly by binding to the NREs.

04030

A POSSIBLE ROLE OF HUMAN IL-1RA 3'-UNTRANSLATED REGION IN MODULATION OF PROTEIN PRODUCTION

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Human interleukin receptor antagonist (hIL-1ra) belongs to the IL-1 family of cytokines. A possible role of hIL-1ra mRNA 3'-untranslated region (3'-UTR) in regulation of IL-1ra synthesis was investigated. Transient expression of chimeric constructs consisting of the luciferase reporter gene followed by hIL-1ra 3'-UTR or its deletion variants was studied in monocyte/macrophage cell lines RAW264.7 and U937. The presence of hIL-1ra 3'-UTR caused significant decrease in the level of reporter gene expression, and this effect was insensitive to addition of LPS. The suppression effect was found to be associated both with the middle and distal segments (relatively to the coding region) of the 3'-UTR sequence. Steady state levels of reporter gene mRNA were not significantly affected by the presence of hIL-1ra 3'-UTR. Inefficient utilization of transcription termination and polyadenylation determinants resulting in accumulation of the unprocessed transcripts in transfected cells could be accounted in part for suppression effect of hIL-1ra 3'-UTR in our experimental system. Translation of synthetic RNA in cell-free system revealed that suppression associated with the middle segment of the hIL-1ra 3'UTR was exerted on the translational level in a cell-specific manner. We conclude that synthesis of the hIL-1ra may be modulated on the translational level, and 3'-UTR of hIL-1ra mRNA may participate in such regulation.

04031

MICROARRAY ANALYSIS OF CYTOKINE AND CELL SURFACE RECEPTOR STIMULATED HUMAN AND MURINE NK CELLS

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Cytokine and cell surface activation of natural killer (NK) cells results in a variety of cellular responses that include increased cytotoxicity, cytokine production, and proliferation. To better understand the molecular mechanisms underlying these responses, microarray analysis was performed on a human NK cell line, NK92 and on in vitro cultured murine NK cells. NK92 cells were treated with IL-2 alone or in combination with IL-12 or IL-18. Of 7075 genes examined 101 genes demonstrated a 2-fold or greater change in expression in at least one of the treatments. These gene transcripts are involved in multiple cellular processes such as cell cycle progression, signal transduction, transcriptional activation, and RNA processing. Relative quantitative RT-PCR of several gene products, has shown that changes in mRNA accumulation of these genes were similar to that predicted by microarray analysis. Murine NK cells were stimulated by cross linking with antibodies to the Ly49D activating cell surface receptor (known to activate IFN-γ mRNA) and the expression of >7000 genes was analyzed. Surprisingly only a relatively small number of genes were found to be strongly induced and the majority of these genes were members of the cytokine/chemokine gene families. Thus, microarray analysis is a reliable method for predicting changes in NK cell gene expression patterns in response to specific extracellular signals.

New/second generation interferons and cytokines I

05001

SECOND GENERATION NON-NATURALLY OCCURRING CYTOKINES: THE NEW FRONTIER

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Over the past two decades recombinant cytokines have emerged as important therapeutic agents that have been successfully applied to the treatment of a wide range of diseases. Examples of these molecules include Interferon (IFN) alpha, beta and gamma, Erythropoeitin, (EPO) Granulocyte Colony Stimulating Factor (GCSF), Granulocyte Macrophage Colony Stimulating Factor (GMCSF), and Interleukin 2 (IL-2). Although the use of cytokines has become widespread, several challenges still exist with respect to treatment optimization. These challenges include poor PK profiles, limited biological activity, and poor therapeutic indices. To ameliorate these problems, several groups have attempted to modify naturally occurring cytokines. These modifications include alteration of the primary amino acid sequences (either by specific changes or by gene shuffling), the addition of polyethylene glycol to the protein, alterations of glycosylation patterns and the production of fusion proteins. Several attempts to engineer cytokines have led to improved therapeutic agents. For example, Interferon-alfacon1 is a second-generation cytokine that was engineered to contain the most frequently occurring amino acids among the non-allelic interferon alpha subtypes. In cell culture models, interferon alfacon1 demonstrates increased potency when compared to naturally occurring type 1 interferons and has been shown to be a more potent inhibitor of the Hepatitis C virus (HCV) in comparative clinical trials with naturally occurring type 1 interferons. Further, the addition of PEG to naturally occurring interferons has led to an improved PK profile with commensurate increases in efficacy with less frequent dosing. Likewise, the addition of PEG to GCSF has improved the PK profile of this cytokine resulting in less frequent dosing. The alteration of the primary amino acid sequence of EPO has led to a protein call NESP that has a modified glycosylation pattern leading to an improved PK profile and less frequent dosing. Finally, several fusion proteins such has IFN alpha hybrids and CSF hybrids have produced promising results that may lead to improved therapeutic agents. Thus, second-generation cytokine have been developed with superior therapeutic properties when compared to the naturally occurring molecules. Several new techniques for selection of novel second-generation cytokines should produce even more potent molecules in the near future.

05012

Evolution of Protein Pharmaceuticals Using DNA Shuffling™

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DNA Family Shuffling™ is a powerful method to improve properties of biological macromolecules based on coupling in vitro recombination of natural genetic diversity with functional selections or screens. This technology has been applied to diverse targets including cytokines, antibodies, enzymes, viruses, RNAs, regulatory elements, enzymatic pathways, and whole genomes. This talk will focus on the applications of DNA Shuffling™ for the production of second generation protein pharmaceuticals with improved potency and selectivity.

05013

PRE-CLINICAL DEVELOPMENT OF PEG-ALPHA INTERFERON FOR TREATMENT OF HEPATITIS-C:

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Poly(ethylene glycol) or PEG has proven to be of great value for a range of biomedical applications. In this lecture we will review the properties of PEG that lead to these applications. Emphasis will be placed on pharmaceutical uses of PEG-proteins, with specific discussion of the attributes of PEGylated alpha-interferon for treatment of hepatitis-C. In this latter case the choice of PEG reagent is critical to the properties of the drug, and therefore a brief presentation of PEG reagents for protein PEGylation will be given.

05004

THE FIRST α HELIX OF IL-2 FOLDS AS AN HOMOTETRAMER, ACTS AS AN AGONIST OF THE IL-2 RECEPTOR β CHAIN AND INDUCES LYMPHOKINE-ACTIVATED KILLER CELLS

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Interleukin-2 (IL-2) interacts with two types of functional receptors (IL-2Rαβγ and IL-2Rβγ) and acts on a broad range of target cells involved in inflamatory reactions and immune responses. For the first time we show that a chemically synthesised fragment of the IL-2 sequence can fold into a molecule mimicking the quaternary structure of an hemopoietin. Indeed, peptide p1-30 (containing amino acids 1 to 30, covering the entire α helix A of IL-2) spontaneously folds into an α-helical homotetramer and stimulates the growth of T-cell lines expressing human IL-2Rβ, whereas shorter versions of the peptide lack helical structure and are inactive. We also demonstrate that this neocytokine interacts with a previously undescribed dimeric form of IL-2Rβ. In agreement with its binding to IL-2Rβ, p1-30 activates Shc and $p56^{lck}$ but unlike IL-2, fails to activate Jak-1, Jak-3 and STAT5. Unexpectedly, we also show that p1-30 activates Tyk-2 thus suggesting that IL-2Rβ may bind to different Jak kinases depending on its oligomerization. At the cellular level, p1-30 induces lymphokine-activated killer (LAK) cells and preferentially activates CD8 low lymphocytes and natural killer cells, which constitutively express IL-2Rβ. A significant IFNγ production is also detected following p1-30 stimulation. A mutant form of p1-30 (Asp20->Lys) which is likely unable to induce vascular leak syndrome, remains capable to generate LAK cells like the original p1-30 peptide. Altogether our data suggest that p1-30 has therapeutic potential.
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05002

IL-22, A NOVEL HUMAN CYTOKINE THAT SIGNALS THROUGH THE INTERFERON RECEPTOR RELATED PROTEINS CRF2-4 AND IL-22R

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We report the identification of a novel human cytokine, distantly related to IL-10, which we term IL-22. IL-22 is a ligand for CRF2-4, a member of the class II cytokine receptor family. No high affinity ligand has yet been reported for this receptor, although it has been reported to serve as a second component in IL-10 signaling. A new member of the interferon receptor family, which we term IL-22R, functions as a second component together with CRF2-4 to enable IL-22 signaling. IL-22 does not bind the IL-10R. Cell lines were identified that respond to IL-22 by activation of STATs, but that were unresponsive to IL-10. IL-22 is produced by activated T cells. In contrast to IL-10, IL-22 does not inhibit the production of proinflammatory cytokines by monocytes in response to LPS nor does it impact IL-10 function on monocytes. Analysis of mice deficient in CRF2-4 has furthered understanding of the roles of IL-10 and IL-22. IL-22 is one of several newly identified molecules related to IL-10, and analysis of the distinct receptor complexes employed by these cytokines reveals a new and unexpected complexity within the class II cytokine receptor family

05003

INTERLEUKIN 21: A NOVEL T CELL-DERIVED CYTOKINE THAT PROMOTES NK CELL EXPANSION AND REGULATES PROLIFERATION OF MATURE B AND T CELLS.

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A novel class I cytokine receptor was identified and shown to be expressed in lymphoid tissues and capable of signal transduction. The full-length receptor was expressed in the IL3-dependent cell line BaF3 in order to create a functional assay for ligand detection and cloning. Conditioned media from activated monkey splenocytes and from activated human $CD3^+$ T cells supported proliferation in the assay cell line. A cDNA library was constructed from activated human $CD3^+$ T cells, and a novel four-helical bundle cytokine was identified using functional cloning. This cytokine, designated IL21, is most closely related to IL2 and IL15. Like IL2 and IL15, IL21 utilizes IL2Rγ in its receptor complex. Quantitative RT/PCR analysis indicates that IL21 is produced almost exclusively by activated CD4+ T cells. Northern analysis and *in situ* hybridization show IL21R expression in lymphoid tissues such as thymus, tonsil, lymph node, PBLs, and spleen. Flow cytometry reveals IL21R expression on resting B cells; quantitative RT/PCR suggests that receptor is also expressed on activated T cells. *In vitro* assays on purified human bone marrow CD34+ cells suggest that IL21 is involved in the proliferation and maturation of natural killer cell populations, in combination with Flt3L and IL15. Assays on human and murine PBMCs show that IL21 supports the proliferation of mature B cell populations in synergy with anti-CD40, inhibits B cell proliferation in response to anti-IgM and IL4, and enhances proliferation of T cells in synergy with anti-CD3.

04004

IFN-κ, a novel type I interferon

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A novel interferon, IFN-κ, was identified as a part of a large scale cDNA sequencing program on the basis of structural similarities to type I interferons. The sequence, derived from a human keratinocyte library, contains an open reading frame of 207 amino acids including a 27 amino acid signal peptide. The mature protein has an identity of approximately 30% to IFN-α, IFN-β, IFN-ω or IFN-δ. Similar to other interferons, IFN-κ gene maps to chromosome 9. Real time PCR analysis of various human cell types revealed the presence of IFN-κ mRNA in keratinocytes, dendritic cells, and in IFN-γ-induced monocytes. As with IFN-β, IFN-κ mRNA expression is up-regulated in keratinocytes by treatment with double stranded RNA, although with different kinetics: the maximal level of IFN-κ expression was observed at 15 hr, while the peak expression of IFN-β was reached at 5 hr. In contrast to IFN-β, INF-κ mRNA expression was also induced by IFN-γ. The gene was expressed in *E. coli* and a recombinant protein of approximately 30 kD was purified. Treatment of human dermal fibroblast with the protein showed that IFN-κ was able to protect the cells from ECMV infection. In addition, IFN-κ had biological activity on human dendritic cells, increasing the expression of activation markers on the cell surface and inducing MIP-1α release. These results, together with the IFN-γ-inducible expression, are suggestive of a potential role for IFN-κ in immunoregulation.

05008

The amino acid residues at position 86 and 90 are important in the antiproliferative activity of human IFN-alphas

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Of the 22 separable forms of human interferon alpha studied in our laboratory, several show remarkable differences in the ratios of their antiproliferative to antiviral activity. To undertand the structural basis for these different activities we previously constructed a series of hybrid interferons in which homologous regions of different interferons were exchanged. These studies identified the regiont from codons 81-95 as very important for antiproliferative activity. In the present work, two mutants in this region were constructed using site directed mutagenesis (SDM), SDM-1 [HY-4(86S →Y)], SDM-2 [HY-4(90N → Y)]. We have found that if S at position 86 was replaced by Y (SDM-1) or N at 90 was replaced by Y (SDM-2) the antiproliferative activity was increased to the level of HY-5. These data suggest that the tyrosines at positions 86 and 90 are important for antiproliferative activity. In order to better understand the contribution that Y at position 86 is playing in the antiproliferative activity, four mutants were constucted by cassette mutagenesis(CM). The Y at position 86 was substituted with different amino acids CM-1[SDM-1(86 Y → D)], CM-2 [SDM-1(86Y→ I)], CM-3 [SDM-1(86Y → K)] and CM-4 [SDM-1(86Y→A)]. We have found that if 86 Y was replaced by I (CM-2) the antiproliferative activity was the same as SDM-1, if 86 Y was replaced by D (CM-1), K(CM-3) or A(CM-4) the antiproliferative activity was decreased. Our results imply that tyrosines at positions 86 and 90 are very important in the antiproliferative activity of IFN-α. Likewise substitution of 86 Y with a hydrophobic amino acid residue (Isoleucine) results in a similar level of antiproliferative activity.

05010

CLONING AND EXPRESSION OF NEW FELINE INTERFERONS ALPHA IN E.COLI

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We have identified several new feline interferons alpha (Fe-IFN-α). PCR was used to amplify the Fe-IFN-α from genomic DNA of the cat lung cells, AKD. Primers flanking the region of the mature protein were chosen according to the published sequence (1). Nested PCR was carried out with primers specific for the mature protein. PCR products of about 536 bp were cloned in an *E. coli* expression vector under a heat inducible promoter. Sequence analysis of nine independent clones revealed changes at the nucleotide and amino acid levels as compared to the previously reported data (1). One clone, identical to the published Fe-IFN-α (1), consisted of 171 amino acids. Six clones contained single nucleotide mismatches; only in three was translation affected (G8V, D46G, L102P). Two clones had multiple amino acid changes and a deletion, resulting in shorter proteins (166 amino acids). High level expression in *E. coli* was observed for all new interferons. Most of the rFe-IFN-α produced by *E.coli* were biologically active as demonstrated by their antiviral activity in AKD cells challenged with VSV. Hu-IFN-αA was used as a standard because it was highly active on feline cells (2). Our results suggest that several of these novel rFe-IFN-α's could be used for therapeutic applications to treat feline viral infections and other diseases.

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05011

POSSIBLE CHANGES IN EXPRESSION OF A CHEMOTAXIN LECT2 mRNA IN MOUSE LIVERS AFTER CONCANAVALIN A-INDUCED HEPATIC INJURY

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Purpose We investigated the involvement of Leukocyte-derived chemotaxin 2 (LECT2) in ConcanavalineA(ConA)-induced hepatic injury in mice.
Materials and Methods ConA was injected intravenously(i.v.) into mice. Z-VAD-FMK was injected i.v. on four occasions after ConA injection. S-GPT activity was measured at various intervals and the excised livers were used to detect DNA fragmentation and to investigate LECT2mRNA and cytokines mRNA after ConA injection.
Results Expression of mRNA of decreased after liver injury induced by intravenous injection of Con A(13 mg/kg) in female BALB/c mice. The expression of LECT2 mRNA completely decreased from 8 to 24 h after the injection, and then gradually increased accompanied with recovery from the hepatic injury. Induction of expression of TNF-□ and IFN-□ mRNAs in the liver was observed after Con A injection. We analyzed whether LECT2 is involved in apoptosis of cells in liver after injection of Con A using a caspase inhibitor Z-VAD-FMK. The inhibitor did not prevent the disappearance of LECT2 mRNA expression, in addition to no inhibition of expression of TNF-□ and IFN-□□mRNAs. This shows LECT2 is not involved in caspase-dependent apoptosis. Hence, Z-VAD-FMK inhibited the increase of serum glutamate pyruvate transaminase (s-GPT) activity and DNA fragmentation in liver tissues after Con A injection. These results suggest that LECT2 may be involved in the regulation of regeneration rather than commitment to apoptosis in the injury. Further, the induction of TNF-□ and IFN-□ may relate to the disappearance of LECT2 mRNA during the injured liver.

05005

IL-2Rβ AGONIST P1-30 ACTS IN SYNERGY WITH IL-2, IL-4, IL-9 AND IL-15: BIOLOGICAL AND MOLECULAR EFFECTS[1]

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From the sequence of human IL-2 we have recently characterized a peptide (p1-30), which is the first IL-2 mimetic described. P1-30 covers the entire α–helix A of IL-2 and spontaneously folds into a α–helical homotetramer mimicking the quaternary structure of a hemopoietin. This neocytokine interacts with a previously undescribed dimeric form of the human IL-2 receptor β chain likely to form the p1-30 receptor (p1-30R). P1-30 acts as a specific IL-2Rβ agonist, selectively inducing activation of CD8 and NK lymphocytes. From human PBMC we have also shown that p1-30 induces the activation of LAK cells and the production of IFN γ Here we demonstrate the ability of p1-30 to act in synergy with IL-2, IL-4, IL-9 and IL-15. These synergistic effects were analyzed at the functional level by using TS1β, as a model, a murine T cell line endogeneously expressing the common cytokine γ gene and transfected with the human IL-2R β gene. At the receptor level, we show that expression of human IL-2Rβ is absolutely required to obtain synergistic effects, whereas IL-2Rα specifically impedes the synergistic effects obtained with IL-2. The results suggest that overexpression of IL-2Rα inhibits p1-30 R formation in the presence of IL-2. Finally, concerning the molecular effects, while p1-30 alone induces the anti-apoptotic molecule bcl-2 we show that it does not influence mRNA expression of c-myc, c-jun and c-fos oncogenes. In contrast, p1-30 enhances IL-2-driven expression of these oncogenes. Our data suggest that p1-30R (IL-2 $R\beta)_2$ and intermediate affinity IL-2 R (IL-2Rβγ), when simultaneously expressed at the cell surface, may induce complementary signal transduction pathways and act in synergy.

05007

The ciliary neurotrophic factor receptor alpha component induces the secretion of and is required for functional responses to cardiotrophin-like cytokine.

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Ciliary neurotrophic factor (CNTF) is involved in the survival of a number of different neural cell types, including motor neurons. CNTF functional responses are mediated through a tripartite membrane receptor composed of two signaling receptor chains, gp130 and the leukemia inhibitory factor receptor (LIFR), associated with a non-signaling CNTF binding receptor alpha component (CNTFR). CNTFR deficient mice show profound neuronal deficits at birth leading to a lethal phenotype. In contrast, inactivation of the CNTF gene leads only to a slight muscle weakness, mainly during adulthood, suggesting that CNTFR binds to an unidentified ligand important for development. Modeling studies of the IL-6 family member cardiotrophin-like cytokine (CLC) revealed structural similarities with CNTF, including the conservation of a site I domain involved in the binding to CNTFR. Co-expression in mammalian cells CLC and CNTFR generates a secreted heterodimeric cytokine displaying activities on cells expressing on their surface the gp130/LIFR complex. Correspondingly, CLC/CNTFR activates gp130, LIFR and STAT3 signaling components, and enhances motor neuron survival. Together, these observations demonstrate that CNTFR induces the secretion of CLC, as well mediating the functional responses of CLC.

05009

A NEW GLYCOSYLATED IFNβ-VARIANT BY PROTEIN-DESIGN SHOWS IMPROVED SOLUBILITY AND BIOAVAILABILITY

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By hydrophobic engineering 8 surface exposed aa (leu and phe) of IFNβ were exchanged by serine. The variant was expressed in CHO-cells and the glycosylated protein was purified to homogeneity. The new IFNβ has a specific antiviral activity of $2x10^8$ U/mg protein, is monomeric and shows an improved solubility. It can be higher concentrated than the unmodified IFNβ and demonstrates a 10 fold enhanced bioavailability in rabbits which is even more pronounced at prolonged time. This new IFNβ is therefore a good candidate for further indications in addition to multiple sclerosis.

16007

CLONING, CHARACTERIZATION AND EXPRESSION OF INTERLEUKIN-19 (IL-19) A NOVEL HUMAN HOMOLOG OF INTERLEUKIN-10 (IL-10)

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We report here identification and cloning of a gene and corresponding cDNAs encoding a novel homolog of IL-10, designated IL-19. The IL-19 gene lies close to the IL-10 gene and their exon/intron structures are similar. The IL-19 gene comprises 5 exons and 4 introns within the coding region of the IL-19 cDNA. However, there are at least two distinct IL-19 mRNA species that differ in their 5'-UTRs, suggesting the existence of an intron in the 5'-UTR of the IL-19 gene. The longer 5'-UTR contains an alternative initiating ATG codon that is in-frame with the coding sequence. Translation from this additional ATG codon would lead to a longer noncanonical leader sequence on the IL-19 protein generating a product that is not as readily as that produced from the IL-19 mRNA encoding the shorter leader sequence. Thus, it is likely that the promoter of the IL-19 gene has complex regulation and can generate two Il-19 forms that differ in their secretion capability. The expression of IL-19 mRNA can be induced in monocytes by LPS-treatment. The appearance of IL-19 mRNA in LPS-stimulated monocytes was slightly delayed compare to expression of IL-10 mRNA: significant levels of IL-10 mRNA were detectable at 2 hours post-stimulation, whereas IL-19 mRNA was not detectable until 4 hours. Thus, unlike LPS-inducible proinflammatory cytokine genes such as TNF-α and IL-1, which are expressed within 1 hour after stimulation, mRNAs for the IL-19 and IL-10 genes are not detecable until 2-4 hours post-stimulation. This delayed induction is consistent with a role for IL-10 and likely IL-19 as feedback inhibitors of proinflammatory cytokine production.Although treatment of monocytes with IL-4 and IL-13 alone did not induce de novo expression of IL-19, these cytokines did potentiate IL-19 gene expression in LPS-stimulated monocytes. IL-19 shares only 21% amino acid identify with IL-10. It does not bind or signal through the canoncial IL-10 receptor complex, suggesting the existence of a IL-19 specific receptors complex which remains to be defined.

Suppressors of cytokine signaling (SOCS)

06005

PHYSIOLOGICAL ROLES OF THE SOCS PROTEINS IN INHIBITING CYTOKINE SIGNALLING

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The Supressor of Cytokine Signaling (SOCS-1) was identified by a functional screen using a retroviral cDNA library for inhibitors of intracellular signaling through IL-6 and LIF receptors in M1 cells. Additional family members were subsequently identified by sequence homology (SOCS-2 to 7) and each contain a central SH2 domain, a conserved C-terminal domain (the SOCS box) and an N-terminal segment of variable length. Both the SH2 and N-terminal domains but not the SOCS box of SOCS-1 were required to inhibit IL-6-induced differentiation in M1 cells. While both SOCS-1 and SOCS-3 could inhibit STAT3 activation their mechanisms of inhibition were different. Phosphopeptide binding analyses revealed that the SOCS-1 SH2 domain bound to JAKS while the SOCS-3 SH2 domain bound with much higher affinity to a sequence corresponding to the gp130 receptor cytoplasmic domain . Using isolated SOCS box peptides or GST-fusion proteins we showed that the SOCS box is necessary and sufficient to interact with elongin B and C complexes which couple bound proteins to the proteasomal degradation pathway. Using mice in which the SOCS box has been selectively deleted from SOCS-1 we have shown that the SOCS box contributes to the capacity of SOCS-1 to inhibit cytokine signalling in a physiological context. Mice in which the entire SOCS-1 gene has been functionally inactivated die within three weeks of birth with fatty degeneration of the liver, monocytic infiltration of several organs and severe lymphoid deficiencies. Much of this pathology can be eliminated by injecting mice with antibodies to interferon-γ or by crossing the mice with interferon-γ knockout mice. SOCS-2-deleted mice are born normally but grow significantly larger than littermates without becoming obese. The unique phenotype of these mice suggests that SOCS-2 normally inhibits signalling from the growth hormone/ insulin-like growth factor axis.

06004

NEGATIVE REGULATION OF CYTOKINE SIGNALS BY SSI-1/SOCS-1; LESSONS FROM DOUBLE KO MICE, SSI-1$^{-/-}$/STAT-1$^{-/-}$, SSI-1$^{-/-}$/STAT-6$^{-/-}$

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SSI-1/SOCS-1 is induced by various cytokines and negatively regulates cytokine signaling. Overexpression studies have revealed that SSI-1/SOCS-1 binds to JAK kinases and inhibits their catalytic activities. The in vivo function of SSI-1/SOCS-1 has been addressed by deriving mice lacking this gene. SSI-1/SOCS-1 deletion results in accelerated apoptosis of lymphocytes, massive fatty change in liver and perinatal lethality with death by 2-3 weeks. It has recently been reported that introducing IFN γ deficiency efficiently rescues SSI-1 KO mice from their disease progression and perinatal lethality, which suggests that SSI-1 is essential for the inhibition of IFN γ in vivo. Interestingly, our recent analysis showed that thymic atrophy and perinatal lethality did not occur in SSI-1/SOCS-1 KO mice also lacking STAT6 gene.

SSI-1-deficient hepatic lymphocytes killed syngeneic wild type (WT) hepatocytes. Depletion of both NKT and NK cell populations reduced their hepatocytotoxicity, while NK cell depletion alone did not, indicating that NKT cells function as effector cells. Administration of anti-CD3 induced lethal hepatitis in pre-onset SSI-1-deficient mice but not in WT or SSI-1/STAT1 or SSI-1/STAT6 double knockout mice, suggesting that simultaneous signaling of multiple cytokines might lead to a biohazardous result. Indeed, SSI-1-deficient T cells showed tyrosine phosphorylation of both STAT1 and STAT6 upon sequential stimulation with IFN-γ and IL-4, whereas WT T cells contained only STAT1 tyrosine phosphorylation. These results suggest a critical role of SSI-1 in homeostasis of the immune system via cross-talk inhibition of cytokine signaling.

06003

SOCS-FAMILY PROTEINS DIFFERENTIALLY REGULATE IL-4-MEDIATED SIGNAL TRANSDUCTION

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SOCS-family proteins were originally identified as cytokine-induced negative regulators of cytokine signaling. We show that SOCS-1 and SOCS-3 inhibit IL-4-dependent Stat6 activation and subsequent gene induction. By contrast, SOCS-2 and CIS upregulate these processes. IL-4 initiates transmembrane signaling through two types of receptor complexes, both of which comprise the IL-4R-α subunit and the associated Jak1 as common essential components. Using an EPOR-IL4Rα chimeric receptor we demonstrate that both SOCS-1 and SOCS-3-mediated downregulation of IL-4 signaling is due to an inhibition of the receptor associated Jak1 activity. The SOCS proteins contain N-terminal region of variable length and primary structure, a central SH2 domain, and a C-terminal conserved motif termed SOCS-box. Using chimeric SOCS proteins we show that the SH2 domains of SOCS-2, SOCS-3 and CIS are functionally redundant in the regulation of IL-4-dependent Jak-Stat signaling. Co-immunoprecipitation and GST pull down experiments demonstrate that SOCS-1 and SOCS-3 interact with latent Jak1 protein further indicating that the function of SH2 domains of SOCS proteins is dispensable. The Pre-SH2 domains confer the specificity of Jak-Stat regulation by the SOCS-family proteins. Importantly, the Pre-SH2 domain of SOCS-3 alone inhibits IL-4 signaling. These results suggest that the Pre-SH2 domains of SOCS proteins play a critical role in the SOCS-mediated differential regulation of IL-4-dependent Jak-Stat signaling.

06002

INTERLEUKIN-4 INHIBITS INDUCTION OF IFN-γ-RESPONSIVE GENES BY INDUCING EXPRESSION OF SUPPRESSOR OF CYTOKINE SIGNALING-1 (SOCS-1).

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IL-4 inhibits the ability of IFN-γ to induce expression of many genes in macrophages. However, the molecular basis for this inhibition has not been defined. We found that IL-4 inhibits IFN-γ-induced activation (tyrosine phosphorylation) of STAT1 in both human monocytes and murine macrophages. Decreased activation of STAT1 resulted in decreased expression of IFN-γ-inducible genes such as FcγRI (CD64) and IP-10. The IL-4-induced inhibition of STAT1 activation could be blocked by actinomycin D, and correlated temporally with expression of the JAK/STAT inhibitory gene, SOCS-1. Moreover, forced expression of SOCS-1 but not SOCS-2 in the murine myeloid cell line, M1, markedly suppressed activation of STAT1 and induction of gene expression by IFN-γ. The ability of IL-4 to induce SOCS-1 gene expression and to inhibit activation of STAT1 was STAT6-dependent. SOCS-1 inhibited activation of STAT1 by blocking the ability of IFN-γ to induce phosphorylation of tyrosine-440 (Y^{440}) on the intracellular domain of the IFN-γ receptor-α chain. Phosphorylation of this tyrosine is an essential step in the IFN-γ signaling cascade because it provides a requisite site for docking and subsequent activation of STAT1. Our findings demonstrate that the ability of IL-4 to inhibit IFN-γ-inducible gene expression is mediated at least in part via its ability to induce expression of SOCS-1. The ability of IL-4 to induce SOCS-1 expression in macrophages may also account for the antiinflammatory activity of this cytokine.

06006

ATTENUATION OF IL-6-TYPE CYTOKINE SIGNALING THROUGH SOCS3 AND SHP2

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The IL-6-type cytokines IL-6, IL-11, LIF, OSM, CNTF and CT-1 signal through the Jak/STAT pathway, which involves the dimerization of the signal transducers gp130, LIF-R or OSM-Rβ, their tyrosine phosphorylation, recruitment of STAT factors and SHP2, translocation of tyrosine- and serine-phosphorylated STAT dimers to the nucleus and binding of STATs to enhancer sequences of respective target genes resulting in transcriptional activation.
Here we describe the functional analysis of the individual cytoplasmic tyrosine residues of gp130 in the context of the full length receptor protein in signaling. We were able to localize an inhibitory tyrosine at position 759. This is consistent with the observation that SHP2 - which binds to Y759 - negatively regulates STAT activation and APP gene induction. Although SHP2 contains two SH2-domains, one SHP2-recruiting phosphotyrosine motif in a single chain of the gp130 dimer is sufficient to mediate SHP2 association to the gp130 receptor subunit and its tyrosine phosphorylation as well as to attenuate IL-6-dependent gene induction. Furthermore, we show that repression of gene induction via Y759 does not require the presence of the SHP2- and STAT-recruitment sites within the same receptor subunit, but within the same receptor complex. The Y759 motif in gp130 also attenuates gene induction mediated by the OSM-R- and LIF-R-complexes, which both contain gp130 as a shared subunit. Finally, we show that tyrosine 759 in gp130 is essential for both SHP2 and SOCS3, but not for SOCS1 to exert their inhibitory activities on interleukin-6-signaling. Besides SHP2 also SOCS3 interacts with a Y(P)759-containing tetradecapeptide of gp130. Taken together, our results suggest differences in the function of SOCS1 and SOCS3 and a link between SHP2 and SOCS3.

06001

PIAS-1 REGULATES THE IFN-γ RESPONSE IN MACROPHAGE CELL LINES

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Macrophage-like cell lines exhibit different profiles of IFN-γ-induced gene expression depending on their maturation stage. We investigated the mechanisms underlying the IFN-γ unresponsiveness in the immature P338.D1 cell line. The binding activity of the signal transducer and activator of transcription-1 (STAT-1) to STAT binding elements (SBE) within the promoter of genes encoding markers of macrophage maturation such as iNOS, FcγRI, and interferon regulatory factor-1 (IRF-1) was reduced in P388.D1 compared to the induction observed in mature RAW264.7 cell. Analysis of the IFN-γ receptor α-chain expression as well as the Jak-1 and Jak-2 kinases activation indicated no difference between the P388.D1 and RAW264.7 cells. Moreover, STAT-1 was phosphorylated and present at similar levels in P388.D1 and RAW264.7 cells. We thus investigated if inhibitory mechanisms were operative in immature cells. Studies on the expression of a negative regulator of cytokine signaling, protein inhibiting activated STAT-1 (PIAS-1), showed that this protein was expressed constitutively at high level in the less-mature P338.D1 cells. IFN-γ stimulated expression of PIAS-1 in RAW264.7, but not in P388.D1 cells. Overexpression of the human Gu binding protein, a PIAS-1 homologue, inhibited the stimulatory effects of IFN-γ on IRF-1 gene transcription in RAW264.7 cells. Conversely, transient transfection of P388.D1 cells with a construct overexpressing STAT-1 was able to rescue the IFN-γ signaling, which reversed the PIAS-1 negative effects. In conclusion, our results indicate that PIAS-1 expression associated with a particular stage of macrophage differentiation accounts for a differential IFN-γ responsiveness.

06013

HUMAN PAPILLOMAVIRUS TYPE-16 E7 ONCOPROTEIN IMPAIRS THE INTERFERON (IFN) RESPONSE

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We have studied the molecular basis for HPV resistance toward IFN action. Initially, we observed that expression of HPV-16 E7 partially abrogated the induction of β-IFN promoter and largely disturbed the activation of the *GBP*-ISRE by IFN-γ. These evidences suggested us that IRF-1 could be a target for this oncoprotein. To verify this, we analyzed the status of the IFN-induced IRF-1 levels under E7-conditional expression using 14/2 BRK cells containing a dexamethasone-inducible HPV-16 E7 gene. Upon E7 induction, a dramatic inhibition of the IRF-1 DNA binding activity was observed in band-shift experiments. However, in W.B. analysis there was not significant change in the levels of IRF-1 expression thus suggesting that E7 disturbs a post-translational event on the IRF-1. Likewise, NFκB but not STAT-1 DNA binding activity was also inhibited under identical conditions. In mutagenesis analysis of E7 , we examined different mutants of this oncoprotein in co-transfections experiments. Data indicated that the CKII phosphorylation site (Ser31/Ser32) on E7 is essential for the inhibitory effect of the ISRE activation. Finally, to verify the role of E7 oncoprotein on the IFN response we transduced the E7 gen into a SLCC cell line very sensitive to the antiproliferative effect of IFN alpha. Examination of the E7-stable transfectants showed a very poor response of these clones to the citostatic effect of IFN-α and an impairment of the $p27^{kip}$ induction when compared to parental cell line. These results describe new E7 targets and could be a part of the mechanisms by which E7 disturbs the IFN signaling pathways and provide an IFN-resistant phenotype.

06014

SODIUM SALICYLATE INDUCES THE EXPRESSION OF THE IMMUNOPHILIN FKBP51 AND BIGLYCAN GENES, AND INHIBITS $p34^{CDC2}$ MRNA BOTH *IN VITRO* AND *IN VIVO*

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One of the mechanisms proposed to explain the anti-inflammatory activity of Sodium Salicylate (NaSal) is based, at least in part, on its ability to inhibit NF-kB activation and inhibition of NF-kB-dependent gene expression. On the other hand, little is known about the ability of NaSal to activate gene expression. By DDRT-PCR, we identified several genes that are modulated upon treatment of mouse fibroblasts with Nasal. From the various cDNA fragments recovered from auto radiograms, we found that NaSal can increase the levels of mRNA for biglycan, the mouse homologue of the human unit, and immunophilin FKBP51. NaSal-induced expression of these genes was time- and dose-dependent. Moreover, the expression of FKBP51 and eIF-3 p47 genes were augmented in vivo, in mice treated orally or I.P with NaSal. Interestingly, the expression of FKBP12 gene, another member of the immunophilin family of proteins was not altered upon in vivo administration of NaSal. We also found that the expression of the p34cdc2 kinase can be inhibited by treating cells with NaSal. The impact this inhibition on cell cycle was evaluated by measuring the content of DNA during the cell cycle. Treatment of cells with Nasal led to a G2/M arrest. By investigating the signaling events that regulate the expression of these genes and their biological activities we can contribute to the understanding of the mechanism of NaSal.

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06012

THE EFFECTS OF IL-6 AND LPS ON THE EXPRESSION OF SOCS BY ENTEROCYTES

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The suppressors of cytokine signaling (SOCS) proteins are a new family of negative regulators of cytokine signal transduction. The SOCS proteins act in a negative feedback loop to suppress signal transduction from cytokine receptors. SOCS gene expression is induced by cytokines both *in vivo* and *in vitro*, and once produced, the SOCS proteins act directly on components of cytokine signaling to shut them off. We have previously shown that IL-6 and LPS stimulation induces IL-8 transcription and production in Caco-2 cells. The purpose of this study was to determine the effect of IL-6 and LPS stimulation of enterocytes on the expression of SOCS and IL-8. Methods: Caco-2 (HTB38, human colonic adenocarcinoma line, were suspended at 2.5×10^5 cells/mL and cultured at 37° C and 5% CO_2 in 12 well tissue culture plates for 7 days. The cells were then stimulated with 100 U/mL IL-6 or 1 μg LPS for 4 hrs. Semi quantitative rtPCR was used to determine mRNA levels. Statistics: ANOVA followed by Tukey's tests.

	$SOCS_1$	$SOCS_2$	$SOCS_3$	IL-8
Media	0.74∀0.02	0.76∀0.02	0.27∀0.03	0.37∀0.01
IL-6	1.09∀0.05*	1.20∀0.02*#	0.881∀0.0*#	1.14∀0.06*#
LPS	1.04∀0.01	0.99∀0.01*	0.35∀0.01	0.73∀0.06

*significantly greater than media
*#significantly greater than LPS

Results: Stimulation with IL-6 or LPS significantly increased the expression of SOCS and IL-8 compared to media. Stimulation with IL-6 significantly increased the expression of $SOCS_2$, $SOCS_3$, and IL-8 compared to LPS. Conclusion: IL-6 and LPS can affect the expression of SOCS enterocytes.

06011

SOCS/SSI-1 is an essential molecule in maintenance of homeostasis of NKT cells via regulating cross-talk inhibition of cytokine signaling in vivo.

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Most hematopoietic cells are exposed to various cytokines at the time of their differentiation and proliferation. However, those multiple signals are screened, integrated and transmitted into cells via various mechanisms. SOCS-1 (SSI-1) is an inhibitor of Jak kinases, and SOCS-1 KO mice come to present a variety of lesions in the thymus, spleen, and liver with aging. But, the hepatic lesions such as fatty degeneration and cell death of hepatocytes were completely eliminated in SOCS-1/STAT1 and SOCS-1/STAT6 double KO mice. This result suggests that simultaneous activation of STAT1 and STAT6 may be responsible for development of the hepatic lesions of SOCS-1 KO mice. Hepatic lymphoid cells of SOCS-1 KO mice showed toxicity against hepatocyte expressing the same MHC class I in vitro. However, removal of NKT cells but not of NK cells from the hepatic lymphoid cells resulted in disappearance of the hepatocyte toxicity. Moreover, peripheral $CD3^+$ T cells lacking SOCS-1 gene no longer showed inhibition of IL-4-induced tyrosine phosphorylation of STAT6 by IFN-γ. Our present study showed that SOCS-1 was a molecule having an important role in cross-talk inhibition of cytokine signaling in vivo. Deficiency of SOCS-1 resulted in disappearance of IL-4 inhibition by IFN-γ, inducing simultaneous intracellular transmission of both IFN-γ and IL-4 signal having antagonistic effects. Consequently, NKT cells expressing both IFN-γ R and IL-4 R might be activated to attain a toxic activity on self-hepatocytes, thereby triggering the hepatic lesions.

06008

Identification of SOCS3 recruitment sites in the murine leptin receptor

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The appetite suppressing hormone leptin, constitutes a negative feedback signal informing the central nervous system of the adipose mass status. Defects in this system result in marked obesity along with a number of other endocrinological disorders. A strong correlation between leptin and adipose tissue mass is often observed in obese patients, which could be explained by leptin resistance. Herein we analyse the recruitment of Suppressors of Cytokine Signalling 3 (SOCS3), a potential mediator of leptin resistance and a broad range cytokine signalling inhibitor, to the activated murine leptin receptor. By using leptin receptor tyrosine mutants in a functional assay based on inhibition of leptin-mediated reporter induction we show that the single tyrosine mutants Y985F and Y1077F still show strong inhibition by SOCS3 while the double mutant Y985/1077F shows absolute lack of inhibition. Binding to both sites was also confirmed by phosphopeptide affinity chromatography. Both Y985 and Y1077 are situated in highly conserved sites. These data indicate that the action mechanism of SOCS3 in leptin signalling is based on both Y985 and Y1077 in the mouse leptin receptor, and not on direct association with activated JAK kinases.

06015

INVOLVEMENT OF JAK3 IN THE IL-2 AND IL-4 DRIVEN EXPRESSION OF CIS IN HUMAN B CELLS

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Cytokines induce a variety of biological responses by binding to specific cell surface receptors, thereby triggering cytoplasmic signal transduction pathways and initiating transcription of genes. The JAK/STAT pathway is one of the major pathways that is used by many cytokines. Although these responses are transient, several molecules have been described that negatively regulate these responses. Recently, a new family of negative regulators of cytokine signal transduction have been described, the so called "suppressors of cytokine signaling"(SOCS). One of the first described members of this family is the "cytokine induced SH2 containing protein" (CIS), which associates with the Il-2 receptor, thereby inhibiting Il-2 dependent signaling. Since Il-2 and Il-4 mediate their effect by signaling through JAK3, we investigated the involvement of JAK3 on CIS expression, by using B cell lines obtained from a JAK3 deficient SCID patient. Northern blot analysis at different timepoints revealed that both Il-2 and IL-4 induce expression of CIS in control B cell lines. However, it was found that CIS expression couldn't be induced by Il-2 and Il-4 in JAK3 deficient B cell lines. These findings suggest, that both Il-2 and Il-4 induced expression of CIS is mediated via a JAK3 dependent signaling pathway.

06007

Apolipoprotein A-I inhibits the production of IL-1β and TNF-α by blocking contact-mediated activation of monocytes by T lymphocytes

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Tumor necrosis factor (TNF-α) and interleukin-1β (IL-1β), essential players in the pathogenesis of immuno-inflammatory diseases, are strongly induced in monocytes by direct contact with stimulated T lymphocytes. To avoid uncontrolled monocyte activation by circulating autoreactive T lymphocytes this cell-cell interaction ought to be inhibited, at least to some extent, in blood. The present study demonstrates that adult human serum (HS) but not fetal calf or cord blood serum displays inhibitory activity towards contact-mediated activation of monocytes by stimulated T cells, decreasing the production of both TNF-α and IL-1β. Fractionation of HS and N-terminal microsequencing as well as electroelution of material subjected to preparative electrophoresis revealed that apo-A1, a negative acute-phase protein, was the inhibitory factor. Functional assays and flow cytometry analyses show that HDL-associated apo A-I inhibits contact-mediated activation of monocytes by binding to stimulated T cells, thus inhibiting TNF-α and IL-1β at both protein and mRNA levels. Furthermore, Apo A-I inhibits monocyte inflammatory functions in PBMC activated by either specific antigens or lectins without affecting cell proliferation. Apo A-I may play an important part in controlling cellular interactions and provide a link between innate and T cell-mediated immune response in inflammation. Furthermore, this novel anti-inflammatory function of apo A-I might lead to new therapeutic approaches in diseases such as rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus and atherosclerosis.

06009

STAT-induced STAT-inhibitor-1 (SSI-1) inhibits not only IFN-gamma signaling but also IL-4 signaling.

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It has been shown that SSI-1/SOCS1/JAB negatively regulates cytokine signaling by inhibiting JAKs. Although SSI-1 in vitro was shown to be induced by various cytokines and inhibit a number of cytokine signaling, recent analyses of SSI-1/IFN-γ double knockout (DKO) mice appear to suggest that SSI-1 in vivo has a specific role in IFN-γ signaling. To study further the role of SSI-1 in vivo, we generated SSI-1/STAT1 and SSI-1/STAT6 DKO mice. We first demonstrated that the injection of anti-CD3 antibody in SSI-1 knockout mice results in remarkable elevation of serum cytokines including IFN-γ and IL-4. We also found that SSI-1-/- splenocytes can easily be polarized both to Th1 and to Th2 phenotype. In addition, the analysis of RAG2 knockout mice reconstituted with SSI-1-/- fetal liver cells suggested that SSI-1-/- lymphocytes in vivo generate enhanced Th2 response after Nippostrongylus brasiliensis infection. These results imply that SSI-1 has an inhibitory role not only in Th1 cytokines such as IFN-γ but also in Th2 cytokines such as IL-4. In line with this, the phenotype of SSI-1 knockout mice including growth retardation, thymic atrophy and early lethality were much less severe in both SSI-1/STAT1 and SSI-1/STAT6 DKO mice. On the other hand, impaired B cell differentiation and decrease in the number of splenocytes was still observed in SSI-1/STAT1 DKO mice but not in SSI-1/STAT6 DKO mice. Taken together, these results suggest that SSI-1 is not a specific inhibitor of IFN-γ, but is a more general inhibitor of cytokines including IFN-γ and IL-4.

06010

Interleukin-9 induces Expression of three Cytokine Signal Inhibitors: CIS, SOCS-2 and SOCS-3 but only SOCS-3 Overexpression Suppresses IL-9 Signaling

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Interleukin-9 (IL-9) is a cytokine preferentially produced by TH2 lymphocytes and active on various cell types such as T and B-lymphocytes, mast cells and hematopoietic progenitors. The IL-9 receptor belongs to the hematopoietic receptor superfamily and its signal transduction mainly involves the JAK/STAT pathway. In this report, we studied the implication of a novel family of suppressors of cytokine signaling (called CIS for Cytokine-Inducible SH2-containing protein, and SOCS for Suppressor Of Cytokine Signaling) in IL-9 signal attenuation. In BW5147 T cell lymphoma, IL-9 induced the rapid expression of CIS, SOCS-2 and SOCS-3 with a peak after 2 hours of stimulation. Using IL-9R mutants, we showed that STAT activation is required for CIS/SOCS induction: CIS and SOCS-2 expression was induced either via STAT1 and STAT3 or via STAT5 but only STAT1 and 3 were involved in SOCS-3 expression. The effect of these three proteins on IL-9 signal transduction was assessed by transient transfection in HEK293 cells expressing the components of the IL-9 signaling pathway and a STAT-responsive reporter construct. These experiments showed that only SOCS-3 is able to inhibit IL-9-induced signal transduction while neither CIS nor SOCS-2 exerted any effect. Stable transfection of CIS and SOCS-3 in BW5147 lymphoma cells showed that only overexpression of SOCS-3 had an inhibitory activity on STAT activation, gene induction and anti-apoptotic activity of IL-9. By contrast, CIS failed to affect the IL-9 response.

Cytokine and interferon gene regulation II

07005

Control of chromatin accessibility for V(D)J recombination by IL-7

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Signals from the IL-7 receptor are critical for early stages in lymphoid development, thus defects in the IL-7 signal transduction pathway lead to severe immunodeficiency in man and mice.
The requirement for IL-7 in vivo is partly attributable to trophic effects, and partly due to effects on V(D)J recombination of immune receptor genes, an event that is stringently regulated at certain early stages in lymphoid development. In order to understand the mechanism by which IL-7 specifically regulates VDJ recombination of the TCRγ locus, we have investigated the role of signal transduction components and the response of the target rearranging genes, including their transcription and specific chromatin alterations, such as histone acetylation. We sought recombination intermediates in vivo and in vitro and analysed the dependence on IL-7 for RAG-mediated cleavage in vitro. We present a model by which IL-7 specifically alters chromatin accessibility of the TCRγ locus, allowing access of the VDJ recombinase.

07006

CHROMATIN REMODELLING ACROSS CYTOKINE GENE PROMOTERS IS AN ESSENTIAL STEP IN TRANSCRIPTION.

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The transcription of an array of cytokine genes, whose products are required to generate an immune response, is dramatically induced following T cell activation. In order to "switch on" the transcription of a gene several molecular steps have to occur. a) The chromatin surrounding the specific gene(s) needs to be disrupted or remodeled, b) Transcriptional activation complexes are assembled on the promoter/enhancer regions of the genes and c) The RNA polymerase enzyme is recruited and transcribes the gene. While there is a large amount of information available regarding the inducible transcription factors required for cytokine gene transcription, there is a paucity of information on other steps such a chromatin remodelling. We are currently examining the role of chromatin for two T cell cytokines. We have found that the IL-2 gene can precisely position a nucleosome across its promoter region and that the presence of this nucleosome occludes the binding of transcription factors. We have also generated evidence, using a novel real-time PCR based assay, that this nucleosome exists *in vivo* in resting primary T cells and is displaced upon activation. By generating transgenic mice with 10kB of DNA spanning the human GM-CSF gene, we find that the wild type transgene is correctly expressed in T cells. Introduction of specific mutations into promoter elements has identified a region of the promoter (an NF-kB/Sp-1 region) that is critical for transcription. Since this mutation has no effect in transient transfections but inhibits transcription in stable cell lines (as well as transgenes), one possible explanation is that the mutation disrupts aspects of chromatin remodeling. This possibility has been verified by the finding that the wildtype but not the mutant transgene can generate a DNaseI hypersensitive site across the promoter upon T cell activation. We are currently using immobilization assays with biotin-labelled DNA promoter fragments to investigate the effect of this mutation on the recruitment of chromatin remodelling activities.

07002

IDENTIFICATION OF AN IL-2 RESPONSIVE ELEMENT IN THE HUMAN IFN-γ PROMOTER

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IFN-γ is a multifunctional cytokine involved in the development of Th1 cells and in cellular responses to a variety of pathogens. Despite concentrated efforts to identify the transcriptional control mechanism(s) by which IL-2 induces IFN-γ mRNA expression, no such genomic regulatory regions have been described. Utilizing DNAse I hypersensitivity analysis, we have identified a potentially new regulatory region distal to previously known elements. We have further characterized this region and found a site that has sequence homology to a consensus STAT5 binding site. Electrophoretic mobility shift analysis utilizing nuclear extracts from IL-2 or IL-12-treated human T cells revealed a strong, unique complex formed with extracts from IL-2 but not with IL-12 stimulated cells. Disruption of the potential STAT site completely eliminated binding of the IL-2-induced complex. Supershift analysis identified both STAT5A and STAT5B in this complex suggesting the formation of STAT5A/STAT5B heterodimers. Moreover, in preliminary experiments, this distal STAT site was linked 5' to an existing luciferase construct containing the core 2.7kb IFN-γ promoter. When transfected into YT cells, the -2.7/STAT construct was responsive to IL-2 stimulation while the -2.7kb vector alone was not. Efforts are currently underway to characterize further the contribution of this site in IL-2-induced transcription of the IFN-γ gene. This report is the first evidence of IL-2-induced STAT proteins interacting with the human IFN-γ promoter.

07004

REGULATION OF PROMOTER ACTIVITY OF INTERFERON REGULATORY FACTOR 7 GENE

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The molecular mechanism by which virus induces expression of the early inflammatory genes has not been yet completely elucidated. Previous studies indicated that the virus mediated transcription of type I interferon (IFN) genes required activation of two members of IFN regulatory factor (IRF) family, IRF-3 and IRF-7 where the expression of IRF-7 was found to be indispensable for the induction of IFNA genes. To determine the factors that regulate expression of IRF-7 gene, as well as its inducibility by type I IFNs, we have isolated and characterized the promoter and first intron of the human IRF-7 gene. This region shows a presence of two potential interferon sensitive response elements (ISRE/IRF-E). However only the ISRE present in the first intron was functional and conferred interferon inducibility in a transient transfection assay. Using a pull down assay with an oligodeoxynucleotide corresponding to this ISRE immobilized to magnetic beads, we have demonstrated that this ISRE binds ISGF3 complex and IRF-1 from the extract of IFN treated cells but not from the untreated cells. In addition, we have identified a cis element in the IRF-7 promoter that upon modification conferred inhibition of both constitutive and inducible expression of IRF-7 gene. We have evidence indicating that this modification can be utilized by some cancer cells, and by virus, to block IRF-7 gene expression. Further details about this modification, its specificity as well as its effect on cells will be discussed.

07003

A NOVEL ROLE FOR PKR IN CONTROL OF mRNA SPLICING

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PKR is expressed constitutively in most cells but induced by viruses, dsRNA and certain cytokines. Like the IFNs, TNF-α exhibits antiviral activity and induces PKR gene expression. PKR may play a key role in TNF-α-induced apoptosis, through phosphorylation of eIF2α and inhibition of translation. We now show that human TNF-α gene expression is regulated by a novel mechanism involving PKR and report a role for the 3'-UTR in control of mRNA splicing. The human TNF-α 3'-UTR, we show, harbors a *cis*-acting element that renders splicing of precursor transcripts dependent on activation of PKR. When this element, designated 2-APRE, is present, splicing in intact cells becomes sensitive to inhibition by 2-aminopurine (2-AP), or by co-expression of transdominant-negative mutant PKR; conversely, increased expression of wt PKR greatly enhances splicing efficiency. Thus, PKR responds as *trans*-acting factor to the 2-APRE. Deletion of the 2-APRE, or its replacement by TNF-β sequences, freed splicing from a dependency on PKR activation while its insertion into the TNF-β 3'-UTR led to acquisition of this control. The 2-APRE is located well upstream of the AU-rich instability motif. RNase sensitivity mapping of 2-APRE RNA reveals a stable, phylogenetically conserved stem-loop structure. The 17-bp 2-APRE duplex is discontinuous yet 2-APRE RNA strongly activates PKR in vitro, inducing eIF2α phosphorylation. Using HeLa cell nuclear extract, we show that splicing is inhibited by 2-AP as well as by anti-PKR Abs, whether directed against the holoenzyme, its kinase domain, or dsRNA-binding domain, showing a requirement for active PKR. Depletion of PKR by these Abs inhibited splicing in vitro. We show that despite the ability of the 2-APRE to activate PKR during splicing, this exonic 3'-UTR element does not affect translation efficiency of the resulting TNF-α mRNA in transfected cells. PKR and the 3'-UTR thus interact during mRNA splicing. These findings demonstrate a novel connection between control of splicing and control of translation, to confer a novel type of regulation on expression of the TNF-α gene.

07001

INTERLEUKIN-1 AND UV-LIGHT INDUCE mRNA STABILIZATION THROUGH DIFFERENT SIGNALING MECHANISMS.

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Post-transcriptional mechanisms play an important role in the control of cytokine gene expression. Expressing mRNAs with a tetracycline-regulated expression system, we have identified an IL-1 activated signal transduction pathway that induces stabilization of mRNAs containing AU-rich regions derived from the 3' untranslated regions of IL-6 and IL-8 mRNAs, as well as the AU-rich elements (ARE) of GM-CSF and c-fos mRNA. That pathway involves p38 MAP kinase and its substrate kinase MAPKAPK2 (MK2). Stabilization of ARE-containing reporter mRNAs was also induced by TPA and ionomycin. IL-1-induced as well as TPA and ionomycin-induced stabilization was inhibited by dominant-negative mutants of the p38 MAP kinase pathway. In contrast, stabilization induced by UV-light, though activating stress-signaling pathways, was hardly affected by these mutants. Furthermore, degradation of luciferase mRNA, which lacks a typical ARE, was not affected by the p38 MAP kinase pathway, but markedly stabilized in response to UV-light. This indicates different transcript selectivities of the two modes of inducing stabilization. Finally, exposure of cells to UV-light markedly increased binding of the Elav-like RNA-binding protein HuR to labeled transcripts *in vitro*, whereas active kinases of the p38 MAP kinase pathway stabilized mRNAs without apparent effect on HuR. These data indicate that UV-light and IL-1 induce mRNA stabilization by different mechanisms.

New/second generation interferons and cytokines II

08006

A NOVEL COMPOSITE CYTOKINE FACTOR WITH BIOLOGICAL ACTIVITIES SIMILAR AS WELL AS DISTINCT FROM IL-12

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08005

THE DEVELOPMENT OF A NOVEL CYTOKINE TO PREVENT SEVERE NEUTROPENIA ASSOCIATED WITH CHEMOTHERAPY

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Neutropenia associated with chemotherapy results in increased risk of infection, dose delays and/or reduced dosing of chemotherapy in cancer patients. Neupogen® (filgrastim) has successfully reduced the duration of neutropenia and its consequences. A new molecule has been tested in over 400 cancer patients in Phase 1-3 studies. This novel cytokine, r-metHuG-CSF-SD/01(SD/01), is comprised of filgrastim conjugated covalently to a polyethylene glycol molecule at the N-terminus. In healthy volunteers, SD/01 was shown to have a prolonged half-life compared to filgrastim and produced dose-related increases in circulating neutrophils and $CD34^+$ cells. Pharmacokinetic and pharmacodynamic results from Phase 2 clinical trials in a variety of malignancies show that one injection of SD/01 per cycle of chemotherapy results in neutrophil support comparable to daily filgrastim. Clearance of SD/01 appears to be mediated predominately via neutrophils, and this mechanism results in "self-regulation" of this molecule in a variety of chemotherapy regimens. In studies of SD/01 in patients with either non-small-cell lung cancer or breast cancer, one injection per cycle of SD/01 at 100 µg/kg resulted in ANC recovery similar to that observed with 8 - 11 daily injections of filgrastim in each of 4 cycles of chemotherapy. SD/01 has been well tolerated and adverse events did not differ in type or severity compared to those sometimes seen with filgrastim. These primarily included musculoskeletal pain and transient increases in serum chemistry values. Following the validation of similar efficacy and safety to daily filgrastim in ongoing pivotal trials, SD/01 has the potential to improve both patient compliance and make neutropenia management more convenient through decreased numbers of injections.

08007

PEGylated Interferon-α2a: Application of Basic Science to the Clinic

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The majority of patients (>80%) with chronic hepatitis C do not respond to "conventional" thrice weekly dosing of interferon-α (IFN-α). Serial quantitative measurements of IFN-α show no measurable serum concentration after 36 hours. When, after one injection its pharmacokinetics are directly related to the viral kinetics of the more "resistent" genotype-1 hepatitis C virus (HCV), one can understand why: an initial decrease of HCV RNA is seen within the first 24 hours followed by a recrudescence during the following 24 hours. This implies that the viral suppression induced with thrice weekly IFN-α in these patients is suboptimal. Pegylation of the IFN-α2a molecule with a large 40 kilodalton branched polyethyleneglycol (PEG) alters its pharmacological properties: rapid and sustained absorbtion, restricted distribution volume, reduced clearance. As a consequence stable serum concentrations can are obtained over a full week after one subcutaneous injection with constant suppressive action on the virus. This has resulted in improved sustained response rates (39%) with once weekly injections of pegylated IFN-α2a (Pegasys®) 180 µg compared to native IFN-α2a (Roferon®-A: 19%) 6 MIU (induction) to 3 MIU (maintenance) thrice weekly for 12 months for patients with chronic hepatitis C enroled in large clinical trials. The application of results from basic science in protein PEGylation to a clinical problem has thus, with one weekly injection, led to a doubling of the rate of sustained responses seen with the native molecule injected three times weekly.

08002

IL-TIF stimulates Acute Phase Reactant production by hepatocytes through IL-10Rß.

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IL-TIF is a new cytokine structurally related to IL-10 and originally identified in the mouse as a gene induced by IL-9 in T cells and mast cells. Here, we report the cloning of the human IL-TIF cDNA, which shares 79% amino acid identity with mouse IL-TIF, and 25% identity with human IL-10. Recombinant human IL-TIF was found to activate STAT-1 and -3 transcription factors in several hepatoma cell lines. IL-TIF stimulation of HepG2 human hepatoma cells upregulated production of acute phase reactants such as Serum Amyloid A, α1-antichymotrypsin and haptoglobin. Although IL-10 and IL-TIF have distinct activities, antibodies directed against the ß chain of the IL-10 receptor blocked the induction of acute phase reactants by IL-TIF, indicating that this chain is a common component of the IL-10 and IL-TIF receptors. Similar acute phase reactant induction was observed in mouse liver upon IL-TIF injection, and IL-TIF expression was found to be rapidly increased after LPS injection, suggesting that this new cytokine contributes to the inflammatory response *in vivo*.

08003

BIOINFORMATIC ANALYSIS OF FIVE GENE SEQUENCES PREDICTED TO ENCODE NOVEL IL-1 - LIKE CYTOKINES

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The IL-1 family of cytokines currently comprises IL-1α, IL-1β, IL-1 receptor antagonist and IL-18. Recently, the family has expanded, with the identification of 5 gene sequences predicted to encode novel IL-1 - like cytokines. Here we propose a standardized nomenclature for the new members, based on that proposed by Smith *et al.* (1), who described 4 of the novel members, naming them IL-1δ, ε, η and ζ. We propose IL-1θ as the name of the 5th novel sequence, having previously been named IL-1H1 (2). We have performed sequence alignments of the family and have identified 3 particular regions of high sequence identity and an additional region of functional importance. We have also carried out secondary structure predictions for each novel family member using up to 9 different prediction programmes. The consensus from these programmes is that the 5 novel members are likely to have a beta trefoil structure similar to that of IL-1α, IL-1β and IL-1Ra. This is supported by tertiary structure prediction which indicates that for all novel members the sequences can be threaded onto the known structure of IL-1β, but importantly not IL-1Ra. A phylogenetic tree of the family has also been compiled and indicates that the novel members are all closely related to each other with classical members more distantly related. The tree for the family indicates a close relationship between IL-1Ra and IL-1δ. Predicted loop structures and the presence of an aspartate at position 148 predict however that IL-1δ may be an agonist rather than an antagonist. Using recombinant IL-1δ and IL-1θ we have been unable to demonstrate any IL-1 - like effects, nor have we found it to block IL-1. Our studies therefore suggest that these novel IL-1 - like sequences may bind novel receptors in the IL-1 receptor superfamily and that IL-1δ may encode for an agonist rather than antagonist.

1. Smith *et al.*, (2000) J Biol Chem 275, 1169-1175. 2. Kumar S. *et al.* (2000) J Biol Chem 275, 10308-10314.

08004

S100 PROTEINS: A NEW CLASS OF CHEMOATTRACTANTS. PROPERTIES OF HUMAN S100A12

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Our studies with the murine S100 calcium-binding protein S100A8 initiated interest in these proteins as novel chemoattractants. Four of this family of 19 are chemotactic. Murine A8 is regulated by LPS, TNF, IL1, IL4, IL10, PGE_2 and corticosteroids. Deletion of the murine A8 gene is embryonic lethal and expression is essential, possibly for trophoblast migration post-implantation.

The human "myeloid-associated" S100 proteins A8, A9 and A12 occur, together with most other S100 genes, within a cluster on chromosome 1q21 (mouse chromosome 3). A8 and A9 are expressed at sites of chronic inflammation including rheumatoid arthritis, cystic fibrosis, Crohn's disease, ulcerative colitis, psoriasis and infection. A12 is anti-filarial and the A8/A9 complex, expressed constitutively in PMN, and known as calprotectin, is anti-microbial. A12 from bovine lung is a ligand for the receptor for advanced glycosylation end products (RAGE). Ligation of RAGE on EC and monocytes induces key inflammatory genes. A12 is implicated in DTH responses and is chemotactic for leukocytes *in vivo*

We show that human A12 is a potent chemoattractant for monocytes (optimal, 10^{-10}M *in vitro*), has weak activity for neutrophils and none on lymphocyte migration *in vitro* and *in vivo*. In contrast, human A8 is inactive *in vitro* but may be protective by scavenging hypochlorite and/or nitric oxide. A12 provoked actin polymerisation and a calcium influx in monocytoid cells of a magnitude comparable to C5a (10^{-8}M). A12 is expressed constitutively in PMN and we demonstrate induction in TNF- and LPS-activated human monocytes. A12+ve cells in synovial tissue of RA patients, include neutrophils at the sublinig and interstitial region and some macrophages in the synovial lining layer. A8 and A12 proteins have been isolated from RA synovial fluid. These proteins may contribute to the pathogenesis of chronic inflammation.

08008

PROTEIN ENGINEERING OF IFNs: STRUCTURE AND FUNCTION

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Abstract not available at time of printing.

Receptor-ligand interactions

09021

DENDRITIC CELL ADHESION MOLECULES AND CYTOKINES

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14007

CORRELATION OF SOLUBLE IFN α/β RECEPTOR IN SERUM OF ALZHEIMER PATIENTS AND THEIR DISEASE STAGE

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Type I interferon (IFN), which includes IFN-α, plays an important role in antiviral and antineoplastic activities as well as cell-mediated immune responses against intracellular pathogens. IFN is synthesized within the immune and central nervous system by T cells and neurons, and displays several functions according to the interactions with specific cell surface receptors. Recently, it has been reported that human serum contains a soluble form of IFN α/β receptors (sIFN α/βR) which inhibits the activity of type I IFN, and a causal relationship between elevated levels of sIFN α/βR and viral infection is speculated in several diseases.

In the present study, serum levels of IFN-α and sIFN α/βR were assessed in 97 patients with Alzheimer's disease (AD), 88 patients with vascular dementia (VD) and 68 age-matched controls. The serum levels of sIFN α/βR were significantly higher in patients with AD as compared with both patients with VD and controls, respectively ($p<0.001$). In patients with AD, there was a significant negative correlation between the serum level of sIFN α/βR and the score on the Mini-Mental State Examination. No significant differences were found in the level of IFN-α in serum.

These findings suggest that sIFN α/βR plays an important role in the pathogenesis of AD, and provide new insights for elucidating the mechanisms of AD.

09004

CORRELATION OF RECEPTOR FOR BINDING ACTIVITY AND ANTI-PROLIFERATIVE ACTIVITY WITH RESIDUAL 86 OF HUMAN IFN-α

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Competitive binding activities of 13 interferon hybrids and mutants of those hybrids were compared with those of human IFN-α2b and IFN-α21a on Daudi cells. The structures of the hybrids are: HY-1 [IFN-α21a (1-75) / IFN-α2c (76-165)], HY-2 [IFN-α21a (1-95) / IFN-α2c (96-165)], HY-3 [IFN-α2c (1-95) / IFN-α21a (96-166)], HY-4 [IFN-α21a (1-75) / IFNα2c(76-81) / IFNα21a (82-95) / IFNα2c (96-165)], HY-5 [IFN-α21a (1-81) / IFN-α2c (82-165)], SDM-1 [HY-4 (86 Ser->Tyr)], SDM-2 [HY-4 (90 Asn->Tyr)], CM-1 [SDM-1 (86 Tyr->Asp)], CM-2 [SDM-1 (86 Tyr->Ile)], CM-3 [SDM-1 (86 Tyr->Ser)], CM-4 [SDM-1 (86 Tyr->Ala)]. The concentration of the components that resulted in 50% inhibition binding ranged from 20 ng/ml to 1000 ng/ml for ^{125}I-IFN-α21a and from 40 ng/ml to 4000 ng/ml for ^{125}I-IFN-α2b. Interferon hybrids HY-1, HY-5, SDM-1, (site directed mutant-1) SDM-2 and CM-2 (cassette mutant-2) compete similarly with IFN-α21a for the ^{125}I-IFN-α21a binding site on Daudi cells, HY-3 showed similar competitive binding activity for ^{125}I-IFN-α2b as IFN-α2b. HY-1 and SDM-1 competed well and CM-1 competed poorly with ^{125}I-IFN-α21a. HY-3 and IFN-α2b competed best with ^{125}I-IFN-α2b and CM-4 competed the least. Each of the interferon hybrids, which competes well with ^{125}I-IFN-α21a had Tyr either in position 86 or 90 and they all have the N-terminal part (amino acids 1-75) of IFN-α21. Only mutant CM-2 (86 Tyr->Ile) had the same competitive binding activity. Mutant CM-1 (86 Tyr->Asp) competed poorly, which could be due to the positive charge Asp. HY-3 competed very well with ^{125}I-IFN-α2b, it has the N-terminal region of IFN-α2c and has Tyr positions 86 and 90. Similar results were obtained in antiproliferative tests. The highest antiproliferative activity was observed for the following mutants: SDM-1, SDM-2 and CM-2. An examination of the hybrids HY-2 and HY-4 showed the poorest antiproliferative activity similar to that of HY-5 and mutants CM-1, CM-3, CM-4. These data suggest that the N-terminal amino acid sequence including the tyrosines at position 86 and 90 may be important for the competitive binding properties and antiproliferative activities of IFNα.

09006

STRUCTURE AND FUNCTION OF A MONOMERIC INTERLEUKIN 10

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A monomeric form of human interleukin 10 (IL-10M1) has been engineered for detailed analysis of IL-10 receptor-ligand interactions. Characterization of IL-10M1 by several biophysical methods reveals that IL-10M1 is a stable α-helical monomer at physiological pH that resembles one domain of the wild type dimer. Incubation of wtIL-10 with soluble form of the IL-10Rα (sIL-10Rα) has previously been shown to generate a complex that consists of 2 wtIL-10 molecules and 4 sIL-10Rαs. In contrast, IL-10M1 forms a 1:1 complex with the sIL-10Rα. Despite the 1:1 interaction, IL-10M1 is biologically active in various cell-based assays. These results indicate that the 1:1 interaction between IL-10M1 and IL-10Rα is sufficient for recruiting the signal transducing receptor chain (IL-10Rβ) into the signaling complex and eliciting IL-10 cellular responses. The high-resolution crystal structure of IL-10M1 with a neutralizing antibody (Ab) has now been determined. The structure of the complex will be presented along with insights into its importance for developing IL-10 antagonists. This work is supported in part by Grants 96008020 from the American Heart Association and NIH-AI36871.

09007

Linkage of host innate defense and adaptive immunity by human antimicrobial peptides: identification of receptors for human β-defensins and LL-37

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Defensins and cathelicidins (LL-37) are two distinct kinds of small antimicrobial peptides produced by phagocytes and those epithelial cells that face the external environment. We have reported that epithelial-derived human β-defensin (HBD) are selectively chemotactic for immature dendritic cells (DC) and memory T cells, whereas neutrophil-derived α-defensin (HNP) was chemotactic for immature DC and resting naïve T cells. We recently found that LL-37 was selectively chemotactic for neutrophils, monocytes, and T cells. Investigation of cells stably transfected with chemokine receptors known to be expressed by both immature DCs and memory T cells revealed that HBD selectively chemoattracted CCR6-transfected cells in a dose-dependent, pertussis toxin-sensitive manner, indicating that CCR6 is a receptor for HBD. The HNP also uses a chemotactic receptor that is in the process of being identified. Screening of cells expressing chemotactic receptors known to be shared by human neutrophils, monocytes, and T cells revealed that LL-37 selectively induced the migration of FPRL1-transfected cells in a dose-dependent, pertussis toxin-inhibitable manner, suggesting that LL-37 utilizes FPRL1 as a receptor. Thus, we have identified human CCR6 and FPRL1 as receptors for HBD and LL-37 respectively. To our knowledge, CCR6 and FPRL1 are the only receptors identified for human antimicrobial peptides. We propose that defensins and LL-37, beside their well-known antimicrobial effects, also mobilize innate host defense and adaptive immunity by recruiting phagocytes, DC and T cells to the site of microbial invasion through interaction with chemotactic receptors.

09002

Chemokine expression by IL-6 and its soluble receptor: Assignment of distinct biological activities to the soluble IL-6 receptor (sIL-6R) isoforms.

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Two forms of the sIL-6R have been identified. One is secreted following differential IL-6R mRNA splicing (DS) and the other is released *via* proteolytic cleavage (PC) of the cell surface IL-6R. Both receptors are structurally related, however the two forms can be distinguished by the presence of 10 unique amino acids at the COOH-terminal of DS-sIL-6R. Although the *in vitro* and *in vivo* release of these two sIL-6R isoforms has been shown to occur *via* independently controlled processes, the significance of each isoform *in vivo* is unknown. Using baculovirus expressed forms of DS-sIL-6R and PC-sIL-6R we have compared the expression profiles of three chemokines (IL-8, MCP-1 & RANTES) by human peritoneal mesothelial cells (HPMC) following stimulation with each sIL-6R isoform. In terms of chemokine expression, data suggest that DS-sIL-6R and PC-sIL-6R may share overlapping but distinct bioactivities. **IL-8**: Both PC-sIL-6R and DS-sIL-6R (1-50 ng/ml) in the presence of 10 ng/ml IL-6 failed to induce IL-8 expression in HPMC, although both suppressed IL-1β induction of IL-8 to a comparable extent (~50-60%). **MCP-1**: PC-sIL-6R and DS-sIL-6R (1-50 ng/ml) in the presence of IL-6 induced a time- and dose-dependent increase in MCP-1 levels. The overall efficacy and potency of the MCP-1 release in response to both sIL-6R isoforms were similar. Antibodies specific for DS-sIL-6R (mAb-2F3) or which did distinguish between to two isoforms (mAb-227) respectively inhibited the DS-sIL-6R-mediated expression and release in response to both isoforms. **RANTES**: Incubation of DS-sIL-6R+IL-6 with HPMC showed a time- and dose-dependent increase in RANTES. No RANTES expression (mRNA or protein) was however observed following stimulation with either PC-sIL-6R + IL-6 or the chimeric sIL-6R-IL-6 fusion protein Hyper-IL-6. RANTES release in response to the [DS-sIL-6R/IL-6] complex was inhibited by mAb-2F3, mAb-227 and by competitive concentrations of PC-sIL-6R (1-50ng/ml). When coupled with the differential *in vivo* expression of DS-sIL-6R and PC-sIL-6R these data suggest that both isoforms may independently contribute to the overall inflammatory properties of sIL-6R. The selective induction of RANTES in response to DS-sIL-6R emphasises that this isoform may have a unique effect on the pattern of leukocyte recruitment observed in inflammatory conditions, such as peritonitis.

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09003

Two different epitopes of the signal transducer gp130 sequentially cooperate upon interleukin-6-induced receptor activation

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Abstract
Cytokines are key mediators for the regulation of hematopoiesis and the coordination of immune responses. They exert their various functions through activation of specific cell surface receptors, thereby initiating intracellular signal transduction cascades which lead to defined cellular responses. As the common signal transducing receptor subunit of at least seven different cytokines, gp130 is an important member of the family of hematopoietic cytokine receptors which are characterized by the presence of at least one cytokine-binding module (CBM). Mutants of gp130 that either lack the Ig-like domain D1 (ΔD1) or contain a distinct mutation (F191E) within the CBM have been shown to be severely impaired with respect to IL-6 induced signal transduction. After cotransfection of COS-7 cells with a combination of both inactive gp130 mutants, signal transduction in response to IL-6 is restored. Whereas cells transfected with ΔD1 do not bind IL-6/sIL-6R complexes, cells transfected with the F191E mutant bind IL-6/sIL-6R with low affinity. Combination of ΔD1 and F191E, however, leads to high-affinity ligand binding. These data suggest that two different gp130 epitopes, one on each receptor chain, sequentially cooperate in asymmetric binding of IL-6/sIL-6R in a tetrameric signaling complex. This implies that formation of a hexameric complex $(IL\text{-}6/sIL\text{-}6R/sgp130)_2$, which has been described to exist in solution, is not necessarily required for IL-6/IL-6R-induced gp130 activation on the cell surface. On the basis of our data a model for the mechanism of IL-6 induced gp130 activation is proposed.

09001

Cytokine-Like Factor-1 associates with Cardiotrophin-Like Cytokine to form a functional heteromeric ligand for the CNTF receptor complex.

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Ciliary Neurotrophic Factor (CNTF) is a cytokine supporting the differentiation and survival of a variety of cell types in the peripheral and central nervous systems. Its tripartite receptor complex consists of a non-signalling α chain, CNTFR, and two signalling β chains, gp130 and the leukaemia inhibitory factor receptor (LIFR). The striking differences in phenotype between CNTF- and CNTFR-deficient mice suggests that CNTFR serves as a receptor for a second, developmentally-regulated ligand. We have identified this factor as a stable secreted complex between cardiotrophin-like cytokine (CLC) and the soluble receptor cytokine-like factor-1 (CLF). CLF expression is required for CLC secretion, with the complex displaying activities only on those cells expressing the functional CNTF receptor. CLF/CLC activates gp130, LIFR and signal transducer and activator of transcription 3 (STAT3) and supports motor neuron survival. Our results indicate that the CLF/CLC complex defines the much sought after second ligand for CNTFR having potentially important implications in central nervous system (CNS) biology. The complex is also a demonstration of a novel control mechanism whereby a cytokine must interact sequentially with two non-signalling "α" chains (CLF and CNTFR) in order to exert its biological effects.

09015

DIFFERENT STRUCTURAL REQUIREMENTS FOR THE ACTIVATION OF GP130 BY INTERLEUKIN-6, ONCOSTATIN M AND LEUKEMIA INHIBITORY FACTOR

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Gp130 is the common signal transducing receptor subunit of interleukin- 6, interleukin- 11, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, and cardiotrophin- 1. Interleukin- 6 as well as interleukin- 11 induce gp130 homodimerization whereas the remaining cytokines lead to the formation of heterodimers of gp130 with a second signal transducing receptor subunit, the leukemia inhibitory factor receptor. The extracellular part of gp130 is predicted to consist of 6 individual domains. In previous studies by site-directed mutagenesis different regions have been identified that are required for the interaction of gp130 with interleukin- 6 and interleukin- 11. Main binding epitopes have been defined to be located in the immunoglobuline-like domain (domain 1) and the cytokine binding module (domains 2 and 3). Here we show that a gp130 mutant lacking domain 1, although unresponsive to interleukin- 6 can still activate STAT in response to leukemia inhibitory factor as well as oncostatin M. Moreover, point mutations in the cytokine binding module of gp130 (F191E and V252D) that severely impair signal transduction in response to interleukin- 6 differentially interfere with gp130 activation in response to leukemia inhibitory factor and oncostatin M. Thus, epitopes involved in binding of cytokines inducing gp130 homodimerization are distinct from those leading to the formation of gp130 / leukemia inhibitory factor receptor heterodimers.

09018

NEW TOOLS TO STUDY APOPTOSIS : MABS AND AN ELISA FOR TRAIL.

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Apoptosis or programmed cell death (PCD) is a genetically programmed cell death essential in the regulation of inflammation and host immune responses. TRAIL (also called Apoptosis inducing ligand 2, Apo2L), a member of the tumor necrosis factor (TNF) family, induces apoptosis in a variety of human tumor cell lines but not in normal cells. Five receptors called death receptors (DR) have been described for TRAIL: DR1, DR2, DR3, DR4 and Osteprotegerin. Only the death receptors DR4 and DR5, containing cytoplasmic death domains, transmit apoptosis.

We describe the development of three murine monoclonal antibodies IgG1 directed against both recombinant and natural TRAIL. These mAbs B-L22, B-S23 and B-T24 are suitable for flow cytometry to detect TRAIL at the cell surface of peripheral blood mononuclear cells stimulated with immobilized CD3 mAbs, soluble CD28 mAbs and IFN-α2a. The activities of the different mAbs in apoptosis are studied by using the myeloid U937 cell line, sensitive to TRAIL mediated PCD. We find different activities for the anti-TRAIL mAbs: B-T24 blocks the TRAIL mediated PCD while B-S23 enhances this process. No effect is observed with B-L22. A TRAIL ELISA detecting recombinant and natural TRAIL in serum, plasma and cell culture supernatants has been generated. After cell activation, TRAIL is detected in cell culture supernatant. The presence of the soluble TRAIL could be the result of shedding or the release of a constitutive or induced intracytoplasmic pool. Investigations to determine the origin of soluble TRAIL are currently performed.

09009

IDENTIFICATION OF IFNAR-1 LIGAND BINDING RESIDUES

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Type I IFNs bind to a common cell surface receptor complex of which two distinct cDNAs have been cloned (IFNAR-1 and IFNAR-2). We are defining the IFN-α2 ligand binding site on IFNAR-1. The extracellular ligand binding region of IFNAR-1 is composed of 4 tandem 100 amino acid subdomains (SD1-SD4). The human and bovine homologues of IFNAR-1 (HuIFNAR-1 and BoIFNAR-1) share 68% amino acid identity and structural similarity, but have distinct ligand binding properties for human IFN-αs: HuIFNAR-1 binds human IFN-αs with low affinity whereas BoIFNAR-1 binds human IFN-αs with high affinity. We previously used bovine/human chimeric receptors to demonstrate the importance of subdomains 2 and 3 for bovine-type high affinity binding of human IFN-αs. These studies have been extended to identify key residues by substituting alanine clusters and single alanine residues throughout the extracellular region of BoIFNAR-1. Several of these mutations have identified residues whose alanine replacement leads to a >80% decrease in binding of IFN-α2. These residues have been located on a 3-dimensional homology model of IFNAR-1, producing a self-consistent map of residues important for the binding of IFN-α2.

09010

Influence of platelet-monocyte complexes (PMC) on the interaction of monocytes with TNF stimulated endothelial cells under flow conditions.

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Monocyte-endothelial cell (EC) interactions are key events in atherogenesis. Selectin receptors on activated EC mediate monocyte rolling under flow conditions before firm adhesion can take place. Moreover, L-selectin-ligand interactions mediate homotypic clustering on monocytes that are already present on the surface. Monocytes, however, also adhere to P-selectin on activated platelets at injured vessel wall or in circulation forming platelet-monocyte complexes (PMC). We studied the influence of PMC on the interaction of monocytes with cultured EC under flow conditions, in a perfusion chamber set-up. Monocytes are obtained by negative selection from MNC cells. As observed by microscopy and FACS analysis, 40% of the isolated monocytes had platelets adhere to their surface. Antibodies against P-selectin inhibited adhesion of these monocytes on 4h α-TNF activated EC by 60%. Removal of PMC (to 5%) from the isolated monocytes decreased adhesion by 30%. The decreased adhesion in both instances coincided with less clustering (70%). Additional presence of antibodies against L-selectin could decrease adhesion and clustering with 30% more. In the presence of PMC, antibodies against L-selectin in contrast, increased clustering with 30%.
In conclusion, activated platelets express P-selectin which enables them to bind to the monocyte surface and form PMC. PMC presence supports monocyte adhesion to EC by increased cluster formation. Homotypic L-selectin dependent interactions of monocytes on EC are most clear in the absence of PMC. Our observations suggest that presence of PMC in circulation is proatherogenic.

09011

COMPLETE IN VIVO NEUTRALIZATION OF HUMAN IL-6 (hIL-6) ACHIEVED BY IMMUNIZATION OF hIL-6 TRANSGENIC MICE WITH THE hIL-6 RECEPTOR ANTAGONIST SANT1

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Neutralization of IL-6 activities represents a possible therapeutic approach to a variety of human diseases. We investigated if the antibody (Ab) response to human IL-6 (hIL-6) induced by immunization with Sant1 (a hIL-6 variant with 7 aminoacid substitutions) neutralized the effects of endogenous IL-6 overexpression in the hIL-6 transgenic mice NSE/hIL-6. In these mice circulating hIL-6 in the range of ng/ml causes a marked decrease in growth rate and in IGF-I levels, making them a model of the growth defect associated with chronic inflammation. NSE/hIL-6 mice show immunological tolerance to the transgene product, therefore representing a suitable system for the evaluation of an anti-IL-6 vaccination protocol. Adult NSE/hIL-6 females were immunized with 100 μg/ml of Sant1 (Sant-Imm) or of hIL-6 (IL6-Imm) in $Al(OH)_3$. Sant-Imm mice developed high titers of Ab to hIL-6 (94000±17088), while IL6-Imm mice did not (10000±9165). After mating with non-transgenic males, the transgenic offspring of Sant-Imm females showed high titers of Ab to hIL-6, acquired by trasplacental transfer, total masking of antigenic hIL-6, and complete correction of the growth defect and of IGF-I levels (results at day 8 of age).

	Offspring of IL6-Imm		Offspring of Sant-Imm	
	Transgenic	Wild-type	Transgenic	Wild-type
Ab to hIL-6 (titer)	6287±1880	ND	41750±16175	ND
Serum hIL-6 (ng/ml)	1.8±1.2	<0.1	<0.1	<0.1
Weight (gr)	2.7±0.4	4.0±0.1	3.9±0.3	4.0±0.2
IGF-I (ng/ml)	70±26	135±13	119±13	122 ± 18

In conclusion, immunization of the NSE/hIL-6 transgenic mice with Sant1 induces an Ab response to hIL-6 which completely neutralizes in vivo the effects of a chronic endogenous overproduction of hIL-6. Vaccination with Sant1 represents a possible therapeutic approach in IL-6-mediated diseases.

09012

EXPRESSION OF TYPE I IFN RECEPTOR ON HUMAN MONOCYTES: CHARACTERIZATION OF DIFFERENTIATION-SPECIFIC POST-TRANSLATIONAL MODIFICATIONS.

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We previously showed that *in vitro* differentiation of human peripheral blood monocytes into macrophages led to the acquisition of a greater sensitivity to the antiviral effect of type I IFN. This enhanced sensitivity correlated with an up-regulation of IFN binding sites at the cell surface and with a re-distribution of the IFNAR-1 subunit from intracellular compartments to the plasma membrane. We now report the biochemical characterization of the type I IFN receptor in this cellular model. Immunoprecipitation experiments showed that upon monocyte differentiation into macrophages the IFNAR-1 subunit underwent to a modification of its apparent molecular weight in SDS-PAGE. Deglycosylation experiments and inhibition of N-glycosylation *in vivo* revealed that the electrophoretic mobility shift was due to a different glycosylation pattern. In contrast to IFNAR-1, the expression and sub-cellular localization of the other receptor chain, IFNAR2-2, was not modulated during *in vitro* differentiation. Its glycosylation is under investigation.
Analysis of the role of N-glycosylation of other cytokine/growth factor receptors has indicated that oligosaccharide side chains may play several important functions, including allowing correct folding of nascent proteins, membrane targeting and anchoring, and modulation of ligand-binding and signal transduction activity. Although the extensive glycosylation of IFNAR-1 has been observed in early studies, its functional significance remains to be determined. We are currently testing the hypothesis that, at least in the monocyte differentiation program, this post-translational modification modulates the cellular sensitivity to type I IFN, by controlling IFNAR-1 membrane localization and/or IFN receptor functionality.

09014

INFLUENCE OF β-ENDORPHIN-LIKE PEPTIDE IMMUNORPHIN ON CYTOKINES AND INTERFERONS BINDING TO MURINE PERITONEAL MACROPHAGES

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In the early 1980s the scientists from the Salk Institute for Biological Studies Julliard et al. (*Science*, 1980, **208**, 183-185), used immobilized antibodies to β-endorphin as affinity absorbents in attempt to isolate this hormone from human placenta extract. A 50 κDa polypeptide was isolated. It was found to be a heavy (H) chain of immunoglobulin G (IgG). Elucidation of the causes of such an effect led to the discovery of a β-endorphin-like sequence in the H-chain. It was found that the 364-377 fragment (SLTCLVKGFYPSDI) was 40% homologous to the β-endorphin antigenic determinant (KSQTPLVTLFKNALKN). An artificial peptide (14 amino acid residues) corresponding to the β-endorphin-like sequence in the IgG heavy chain was synthesized by Houck et al. (*Science*, 1980, **207**, 78-79) and found to interact with opioid receptors on rat brain cells. Our research group has synthesized and studied the β-endorphin-like decapeptide (H-SLTCLVKGFY-OH) corresponding to amino acid residues 364-373 of IgG H-chain and referred to as immunorphin (*Immunol. Lett.*, 1996, **49**, 21-26). The results of the study indicate that immunorphin has a potent (>100 times as potent as tuftsin) immunostimulatory activity *in vitro*: the peptide activates natural killers, splenic T- and B- lymphocytes as well as lung and peritoneal macrophages of mice. It has been found that the peptide inhibits in a competitive manner, the binding of ^{125}I-labeled β-endorphin to naloxone-insensitive β-endorphin receptors on mouse peritoneal macrophages. At the same time immunorphin (1nM0 increases the binding affinity and receptors density of [^{125}I]HuTNF-α, [^{125}I]HuIL-1β, [^{125}I]HuIFN-γ and [^{125}I]HuIL-6 on the surface of these cells. On the whole, the results obtained together with previously reported findings that IL-1β, IL-6, TNF-α и IFN-γ expression by macrophages is elevated at the presence of immunorphin, suggest that these proteins and their receptors participate in the molecular mechanism of immunorphin action.

09016

INHIBITION OF IL-6/gp130 COMPLEX ACTIVITY BY SYNTHETIC PEPTIDES.

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The interleukin-6 (IL-6) cytokine is vital for tissues and organs and plays an important role in proliferation and cancer. IL-6 signal-transducing complex is composed of the ligand IL-6, the IL-6 receptor and at least two gp130 molecules. Transmembrane gp130 is a signal-transducing subunit shared by all the IL-6 family molecules.
The purpose of this study is mainly to clarify the particular sites of IL-6/gp130 complex that are involved in the proliferation of myeloid cells. This has been attempted by using synthetic complementary peptides that have the ability to modify this biological activity. The later was tested by B9 myeloid cell proliferation test.
The experimental results showed that some peptides which are related to the extracellular part of gp130 protein, were found to inhibit IL-6/gp130 complex activity in micromolar concentration and in a dose dependant mode.
These peptides can possibly be used as potential modifiers of gp130 activity in various pathological disorders associated with IL-6 family molecules.
Therefore, it could be assumed that the synthesis of complementary peptides can be a valuable tool for designing lead inhibitory peptide modifiers of various biological molecules such as cytokines and/or their receptors.

09017

STUDY OF CHIMERIC TYPE I INTERFERON RECEPTORS AND THEIR USE IN THE DEVELOPMENT OF BIOASSAYS.

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A series of chimeric receptors, consisting of the extracellular (EC) domains of several cytokine receptors and of the transmembrane (TM) and intracellular domains (IC) of the type I interferon receptor was constructed and tested for functionality in 2fTGH 6-16SEAP cells. These cells stably express the genes for *gpt* and SEAP under transcriptional control of the interferon-inducible 6-16 promoter, allowing quantification of activation of the interferon signalling pathway. As such, clustering of the IFNaR2 IC domains proved to be functional after Epo-induced dimerization of the EpoR/IFNaR2 chimeras, but not after IL-5-induced IL-5Rα/IFNaR2 + βc/IFNaR2 clustering. IL-5 however, induced 6-16 promoter activation via heteromeric clustering of IL-5Rα/IFNaR2 + βc/IFNaR1 chimeras. These results indicate that clustering of the IFNaR2 chains is not sufficient for activation and that topological requirements may play an additional role. Since it is known that, IFNα/β differential signaling is transmitted via the same receptor components, we are now investigating the possible role of topology in differential IFNα/β activity. In addition, these cells allow very sensitive detection of Epo and IL-5. Chimeric receptors harbouring the EC domains of other cytokine receptors such as TNF-R55, EGFR and IFN-γR1 and 2 were also responsive to their cognate ligands. As such, using the 2fTGH cells as a central, sensitive core, an uniformed series of sensitive bioassays can be developed.

09019

PREPARATION OF ^{32}P-LABELED HUMAN RECOMBINANT INTERLEUKIN 11 FOR SUCCESSFUL RECEPTOR BINDING STUDIES *

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Human interleukin 11 (hIL-11) is a multi-potential cytokine which is involved in numerous biological activities such as hematopoiesis, osteoclastogenesis, neurogenesis and female fertility. Its physiological functions have been largely documented, but little is known about its binding to cell membrane receptors, mainly because of a loss of biological activity and binding when using conventional radioiodination techniques. Here we report the production of a new radioactively labeled form of human recombinant IL-11 useful for receptor binding studies. In this molecule, the first ten amino acids (P-G-P-P-P-G-P-P-R-V) of the mature hIL-11 have been replaced by a specific phosphorylation peptide (G-R-R-A-S-V-A). Its biological activities and immunoreactivities were similar to those of wild-type human recombinant IL-11. This new molecule (P-ΔIL-11) was labeled with [γ-^{32}P]ATP using bovine heart protein kinase. The phosphorylation was specific and confirmed by phosphatase treatment. The resulting ^{32}P-labeled P-ΔIL-11 (^{32}P-ΔIL-11) with radiological specific activity (~100,000 cpm/ng of protein) retains full biological activity. The binding of ^{32}P-ΔIL-11 to Ba/F cells stably transfected with plasmids encoding for human IL-11 receptors α and β chains (IL-11Rα and gp130) is specific and saturable, with a Kd in the nanomolar range as determined from Scatchard plot analysis. Availability of this new ligand should be useful for further studies on IL-11R.

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09020

Neutralising Monoclonal Antibodies can potentiate Interleukin-5 signaling

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IL-5 is a major determinant in the survival, differentiation and effector-functions of eosinophils. It mediates its effect upon binding and activation of a membrane bound receptor, which is composed of a ligand-specific α-chain (IL-5Rα) and a β-chain (β_c), which is shared with the receptors for IL-3 and GM-CSF.
We have generated and mapped the epitopes of three monoclonal Abs directed against this cytokine: the strong neutralising mAbs 5A5 and 1E1, and the very weak neutralising mAb H30. We found that H30 as well as 5A5 can increase proliferation above the level induced by hIL-5 alone, at every sub-optimal hIL-5 concentration analysed. Using F_{AB}-fragments we could show that this effect is dependent on Ab-mediated cross-linking of IL-5R complexes. Absence of potentiation with monomeric and the asymmetric single chain mutant wt/A5 further supports this hypothesis. However, potentiation is only observed on cell-lines expressing a hybrid human/mouse IL-5Rα-chain, most likely indicating the topological constraints of the observed effect. Potentiating effects by neutralising mAbs directed against IL-5, and possibly other dimeric cytokines including IL-10, PDGF, IFN-γ, EGF, warrant a careful evaluation prior to their in vivo application.

09008

THE ROLE OF THE IL-13 RECEPTORS α1 AND α 2 IN IL-4 AND IL-13 SIGNALLING PATHWAYS.

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IL-4 and IL-13 are pleiotropic cytokines secreted by activated Th2 cells. IL-13 immuno-regulatory activities partially overlap those of IL-4. Like IL-4, IL-13 can induce IgE and IgG4 synthesis in activated B cells.

The receptor specific for IL-4 is a combination of the IL-4Rα chain and the common γc chain. For IL-13, two binding proteins have been cloned : IL-13Rα1 and IL-13Rα2. IL-13Rα1 becomes a high affinity receptor when co-expressed with IL-4Rα, while IL-13Rα2 alone binds to IL-13 with a higher affinity than IL-13Rα1. However, IL-13Rα2 has a short intra-cytoplasmic domain and makes it unlikely to have signaling capacity.

A panel of monoclonal antibodies against IL-13α1 or IL-13Rα2 has been generated in our laboratory. These antibodies recognize the respective transfected CHO cells and certain cell lines or cell types. We have set up a biological assay using the IL-6 production by monocytes after lipopolysaccharide (LPS) stimulation. In the presence of IL-4 or IL-13, this IL-6 production is decreased.

The influence of the anti-IL-13Rα1 and anti-IL-13Rα2 monoclonal antibodies in this biological assay will be presented and may elucidate the contribution of the IL13Rα1 and IL13Rα2 in IL-4 and IL-13 signaling.

09013

THE IL-6-TYPE CYTOKINE SIGNAL TRANSDUCER GP130: A NOVEL MECHANISM OF RECEPTOR ACTIVATION BY MONOCLONAL ANTIBODIES

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The transmembrane glycoprotein gp130 belongs to the family of hematopoietic cytokine receptors. It represents the common signal transducing receptor component of the so-called interleukin-6-type cytokines. For several cytokine receptors including gp130 it has been shown that receptor activation can not only be achieved by the natural ligand but also by single monoclonal antibodies raised against the receptor ectodomain. These findings have been interpreted in a way that dimerization of cytokine receptors is sufficient for receptor activation. Here we show that the recently described gp130-activating antibody B-S12 actually consists of two different monoclonal antibodies. By subcloning of B-S12 the monoclonal antibodies B-S12-A5 and B-S12-G7 were obtained. The individual antibodies are biologically inactive, in combination they exert B-S12-like activity on hepatoma cells. On Ba/F3 cells stably transfected with gp130 a combination of B-S12-G7 with another monoclonal gp130 antibody, B-P8, is required to stimulate proliferation. Using gp130 deletion mutants we show that all three antibodies map to domains 2 and 3 of gp130 which constitute the cytokine binding module. The individual antibodies inhibit activation of the signal transducer by IL-6 and interfere with binding of IL-6 to gp130. Interestingly, the combination of B-S12-G7 and a Fab-fragment of B-P8 retains biological activity. We conclude from our data that (i) the monoclonal antibodies activate gp130 by mimicking the natural ligand and (ii) enforcement of gp130 dimerization is not sufficient for receptor activation but additional conformational requirements have to be fulfilled.

Signal transduction I

10005

NOVEL CROSS-TALK MECHANISM BETWEEN TYPE I AND TYPE II INTERFERON RECEPTORS

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The interferons (IFNs) are classified into two types of interferons, IFN-α/β and IFN-γ, which are structurally unrelated and known to transmit signals through distinct receptor complexes, IFNAR and IFNGR, respectively. Notably, stimulation of IFNAR and IFNGR, each consisting of two subunits (IFNAR1 and IFNAR2, or IFNGR1 and IFNGR2) commonly results in activation of the Stat1 transcription factor. These IFN-α/β and IFN-γ systems have been extensively studied as paradigms of cytokine signaling, however, the issue has not yet been rigorously addressed as to whether they share any functional aspects in the signaling process. In the present study we report on a novel aspect of IFN-γ signaling that is dependent on the IFN-α/β system. In fact, the IFN-γ-induced activation of the Stat1 and ISGF3 transcription factors as well as antiviral response is impaired in IFNAR1-deficient cells. Similar defect was observed in cells deficient in IFN-α/β production, indicating that the full IFN-γ response is contingent on a weak IFNAR stimulation by spontaneously produced IFN-α/β. We also demonstrate that the intracellular tyrosine residues of IFNAR1, which undergo phosphorylation by a weak ligand stimulation, provide niches for efficient Stat1 dimerization upon IFNGR stimulation. We also provide evidence for a physical association between IFNAR1 and IFNGR2, which occurs exclusively at the caveolar membrane domains on the cell surface. Furthermore, the Janus kinases are also localized to these domains. Thus, our present findings provide not only a novel cross-talk mechanism between these two IFN receptors but also a new, putative basis for understanding the cytokine receptor signaling mechanism on the cell surface, which may apply to other cytokines.

10001

Identification of a Novel Physiologically-Relevant Stat1-Independent IFNγ Receptor (IFNγR) Signaling Pathway.

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The current model of IFNγR signaling suggests that IFNγ induces biologic responses in cells by activating Jak1, Jak2 and Stat1. The physiologic relevance of this model was established by generating and characterizing mice that lack the genes for each of these signaling proteins. To assess whether Stat1 is required for all the IFNγ's effects on cells we compared the activity of IFNγ on the growth of primary bone marrow cells (BMC) derived from wild type (WT) versus Stat1 deficient ($Stat1^{-/-}$) mice. Surprisingly IFNγ provided a growth/survival/differentiation signal for $Stat1^{-/-}$ BMC and also enhanced growth induced by CSF-1. In contrast, IFNγ had an antiproliferative effect on WT BMC. To explore whether IFNγ exerted its function on $Stat1^{-/-}$ BMC by regulating gene expression, we employed both subtractive cloning approaches (RDA) and gene array technologies. A large number of genes were identified that were regulated by IFNγ in cells lacking Stat1. The validity of this observation was confirmed by monitoring changes in expression of the corresponding proteins. Regulation of at least some of these genes still requires the IFNγ receptor and Jak1. Thus there exists at least one alternative Stat1 independent pathway of signaling through the IFNγ receptor. The physiologic relevance of this pathway was defined using in vivo models of viral infection.

10003

IFNα/β promotes cell survival by activating NF-κB. CH Yang, A Murti, SR Pfeffer, JG Kim, & LM Pfeffer. University of Tennessee Health Science Center, Department of Pathology, Memphis, TN, USA

IFN plays a critical role in the host defense by modulating the expression of various genes via the IFN-dependent tyrosine phosphorylation of STAT transcription factors. We show that IFNα/β activates another important transcription factor, nuclear factor kappa B (NF-κB). NF-κB DNA-binding activity was induced by multiple type-1 IFNs, and was promoted by IFN in a diverse group of human, monkey, rat and murine cells. Human IFN promoted NF-κB activation in murine cells that express the human IFNAR1 signal-transducing chain of the IFNα/β receptor. NF-κB normally exists as a dormant cytoplasmic complex due to the binding of inhibitory IκB proteins. IFN receptor signaling rapidly results in the serine phosphorylation and degradation of IκBα, leading to the stimulation of NF-κB DNA binding and transcriptional activity. IFN promotes IκBα serine phosphorylation and degradation, and stimulates NF-κB DNA binding and transcriptional activity. Super-repressor forms of IκBα inhibit IFN-mediated NF-κB activation and IκBα degradation. Importantly, IFN promotes cell survival by protecting cells against a variety of pro-apoptotic stimuli, such as virus infection, antibody-mediated crosslinking, and IFN itself. Super-repressor forms of IκBα block IFN-promoted cell survival, enhancing apoptotic cell death. We conclude that NF-κB activation is integrated into an IFN signaling pathway through the IFNAR1 chain of the IFNα/β receptor that promotes cell survival.

10002

INTERFERON REGULATORY FACTOR (IRF) SUBCELLULAR LOCALIZATION IS DETERMINED BY A BIPARTITE NLS IN THE DNA BINDING DOMAIN AND INTERACTION WITH CYTOPLASMIC RETENTION FACTORS

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The transduction of Type I interferon signals to the nucleus relies on activation of a protein complex, ISGF3, involving two STAT proteins, STAT1 and STAT2, and the IRF protein, p48/ISGF3γ. The STAT subunits are cytoplasmically localized in unstimulated cells and rapidly translocate to the nucleus of interferon stimulated cells, but the p48/ISGF3γ protein is found in both the nucleus and the cytoplasm regardless of IFN stimulation. Here we demonstrate that p48 is efficiently and constitutively targeted to the nucleus. Analysis of the subcellular distribution of green fluorescent protein-p48 fragments indicates that p48 contains a novel bipartite nuclear retention signal within its amino terminal DNA binding domain. This signal is preserved in two other IRF proteins involved in immune responses, ICSBP and IRF-4. Mutations to clustered basic residues within amino acids 50-100 of p48 or IRF-4 disrupt their nuclear accumulation, and DNA binding ability is not required for nuclear targeting. This is the first example of a nuclear localization signal for any ISGF3 component, and a novel function for the IRF DNA binding domain. We also demonstrate that the nuclear distribution of p48 is dramatically altered by co-expression of the STAT2 protein, indicating that STAT2 forms a cytoplasmic complex with p48, overriding the intrinsic p48 nuclear targeting. Retention by STAT2 is reminiscent of the cytoplasmic retention of NFkB by IkB and may serve to regulate the activity of free p48 and/or guarantee that cytoplasmic pools of pre-associated STAT2:p48 are available for rapid activation of the IFN response. These findings suggest analogous mechanisms may exist for regulating the distribution of other IRF proteins.

10004

A novel Interferon-γ stimulated gene regulatory pathway mediated by CCAAT/Enhancer binding protein-beta (C/EBP-β) and Extracellular signal Regulated Kinases (ERK)

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We have previously identified a novel Interferon responsive *cis*-acting enhancer element called γ-IFN activated transcriptional element (GATE) in the promoter of murine ISGF3γ (p48) promoter. GATE is distinct from the known IFN stimulated elements and binds to novel transacting factors. We have identified the GATE binding factors from an IFN-γ stimulated murine macrophage cell line and isolated 3 different cDNAs. Among these is a gene coding for the transcription factor, CCAAT/enhancer binding protein-β. C/EBP-β binds to GATE and induces gene expression. A mutant C/EBP-β interferes with the IFN-γ stimulated transcription from ISGF3γ (p48) promoter. Other members of the C/EBP family do not cause these effects. Interestingly, the expression of C/EBP-β is induced by IFN-γ. In addition the stimulation of basal transcription, IFN-γ treatment further augments C/EBP-β dependent gene expression. These data identify the involvement of a kinase in this pathway. Here we describe a new IFN-γ stimulated pathway that operates C/EBP-β regulated gene expression independent of JAK1. We show that extracellular signal regulated kinases (ERK) are activated by IFN-γ to stimulate the C/EBP-β dependent expression. Sustained ERK activation directly correlates with C/EBP-β dependent gene expression in response to IFN-γ. Mutant MKK1 or its inhibitors or mutant ERK suppress IFN-γ stimulated gene induction through GATE. Ras and Raf activation are not required for this process. Furthermore, IFN-γ induced Raf-1 phosphorylation negatively correlated with its activity. Interestingly, C/EBP-β- induced gene expression requires STAT1 but not JAK1. A C/EBP-β mutant, lacking the ERK phosphorylation site, fails to promote IFN-stimulated gene expression. Our data establish a link between C/EBP-β and IFN-γ signaling through ERKs.

10006

Similarities and differences in signaling pathways that respond to IL-18 and IL-1

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Interleukin-18 (IL-18) a pleitropic cytokine produced by activated macrophages, plays significant roles in the immune response, including the induction of the cytokines IFNγ and IL-2, the enhancement of NK cell activity, and the potentiation of Th1 differentiation The intracellular signal transduction pathways by which IL-18 exerts its actions have not been well defined. The IL-18 receptor subunits and IL-18 itself have tertiary structure's similar to those of their IL-1 counterparts, suggesting that the IL-1 and IL-18 signaling pathways might share similar or identical components. Here we use mutant 293 cell lines that are unresponsive to IL-1 to demonstrate that IL-1 and IL-18 signaling pathways are indeed similar, but not identical. An IL-1-unresponsive mutant cell line that lacks an unknown component retains responsiveness to IL-18, indicating that the pathways are different. The IL-1 receptor-associated kinase (IRAK), essential for IL-1 signaling, is also necessary for the activation of both NF-κB and JNK in response to IL-18. Finally, we demonstrate that the death domain, but not the kinase activity of IRAK is necessary for NF-κB activation in response to IL-18, just as for IL-1.

10034

Involvement of p38 Mitogen-activated Protein Kinase Signaling Pathway in Osteoclastogenesis Mediated by Receptor Activator of NF-κB Ligand (RANKL)

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The receptor activator of NF-κB ligand (RANKL) induces osteoclast differentiation from bone marrow cells in the presence of macrophage colony-stimulating factor (M-CSF). We found that treatment of bone marrow cells with SB203580 inhibited osteoclast differentiation *via* inhibition of the RANKL-mediated signaling pathway. To elucidate the role of p38 mitogen-activated protein (MAP) kinase pathway in osteoclastogenesis, we employed RAW264 cells which could differentiate into osteoclast-like cells following treatment of RANKL. In a dose-dependent manner, SB203580 but not PD98059 inhibited RANKL-induced differentiation. Among three MAP kinase families tested, this inhibition profile coincided only with the activation of p38 MAP kinase. Expression in RAW264 cells of the dominant negative form of either p38α MAP kinase or MAP kinase kinase (MKK) 6 significantly inhibited RANKL-induced differentiation of the cells. These results indicate that activation of the p38 MAP kinase pathway plays an important role in RANKL-induced osteoclast differentiation of precursor bone marrow cells.

10035

Three-dimensional Structure of the Type I Interferon Receptor Signalling Complex: Modelling in the Large

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With the recent publication of the FERM structure (Moesin) and the recognition of sequence homologies between RAS interactve proteins and the intracellular components of the interferon receptor subunits, suitable templates are now available for the three dimensional homology modelling of all the components of the TypeI Interferon receptor transduction complex. While the finer refinements of docking are still beyond reach, 3 D modelling in the large is feasible. We present molecular models of the complex showing how orientational organization on the inside of the membrane is likely to affect that on the exterior and vice versa for a variety of Type I interferons.

10036

ROLE OF SRC KINASES IN CHEMOATTRACTANT- AND CYTOKINE-MEDIATED NEUTROPHIL FUNCTION

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Neutrophils play a critical role in the host defence to microbial pathogens. Stimulation of these cells induces multiple responses, including cell adhesion, migration, secretion, phagocytosis and generation of reactive oxygen species. Tyrosine phosphorylation is thought to be critical in the regulation of neutrophils and members of the Src-family of tyrosine kinases have recently been shown to be regulated in activated granulocytes. We have utilised a specific Src-kinase pharmacological inhibitor, PP1, to evaluate the role of Src-kinases in cytokine/chemoattractant signalling regulating neutrophil function. PP1 was found to potently inhibit activation of the Src kinase Lyn in human peripheral blood neutrophils. Investigation of Src in relation to several signal transduction pathways, showed that PP1 inhibits PKB phosphorylation but did not inhibit the activation of ERK and p38 MAP kinases upon fMLP and GM-CSF stimulation of neutrophils. Furthermore, pre-treatment of neutrophils with PP1 resulted in a strong inhibition of fMLP-induced superoxide production, suggesting a role in the regulation of the NADPH-oxidase complex. Analysis of fMLP-induced actin reorganisation in neutrophils in suspension, revealed a reduction in the extent of F-actin generation in cells pre-treated with PP1. Pre-treatment of neutrophils with PP1 also showed a partial inhibition of GM-CSF induced chemokinesis, while PP1 did not inhibit fMLP induced chemotaxis. Finally, addition of PP1 reversed the cytokine-mediated rescue of freshly isolated cultured human neutrophils. These data implicate the Src-family of tyrosine kinases as critical upstream mediators regulating diverse neutrophil effector functions.

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10029

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TITLE: GRANULOCYTE COLONY-STIMULATING FACTOR INDUCES ERK5 ACTIVATION WHICH IS INVOLVED IN THE REGULATION OF CELL PROLIFERATION AND SURVIVAL

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Abstract

Granulocyte Colony-stimulating factor (G-CSF) regulates the proliferation, differentiation and survival of granulocytic progenitor cells. Treatment of cells with G-CSF has been shown to activate multiple signal transduction pathways. We show here that Erk5, a novel member of the MAPK family, and its specific upstream activator MEK5 were activated in response to incubation of cells with G-CSF. Different from other members of the MAPK family including Erk1/2, JNK and p38, maximal activation of Erk5 by G-CSF required the carboxy terminal region of the G-CSF receptor, a region that has previously been shown to be required for induction of granulocytic differentiation. Genistein, a specific inhibitor of protein tyrosine kinases, blocked G-CSF induced Erk5 activation. In contrast, suppression of protein kinase C (PKC) activity increased G-CSF-mediated activation of Erk5 and MEK5 whereas stimulation of PKC activity markedly inhibited activation of the two kinases by G-CSF. The proliferation of BAF3 cells in response to G-CSF was inhibited by expression of a dominant negative MEK5 but potentiated by expression of a constitutively active MEK5. The constitutively active MEK5 also dramatically increased the survival of BAF3 cells cultured in the absence or in low concentrations of G-CSF. Together, these data implicate Erk5 as an important signaling component in the biological actions of G-CSF.

10038

ACTIVATION OF p38 MAPK BY IL-1 — A SIGNAL TRASDUCTION PATHWAY THAT INVOLVES MyD88, IRAK1/2, TRAF6, RAS AND RAP.

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Previously we have shown that IL-1 can activate p38 MAP kinase via the small G protein Ras, and that this activity can be down regulated by another small G protein Rap, consistent with Rap antagonising Ras function (1). Here we have further investigated the role of Ras and Rap in p38 activation by IL-1. Transfecting cells with constitutively active forms of the known IL-1 signaling components MyD88, IRAK-1, TRAF-6, and MEK3 activated p38. Dominant negative forms of MyD88, IRAK-1 and TRAF-6 were then tested and were found to inhibit activation of p38 by IL-1. Dominant negative RasN17 blocked the effect of the active forms of all but MEK3, indicating that Ras lies downstream of TRAF-6 but upstream of MEK3 on the pathway. Furthermore, the activation of p38 caused by overexpressing active RasVHa, could not be inhibited using dominant negative mutants of MyD88, IRAK1 or 2, or TRAF6. In the same manner, the inhibitory effect of Rap on the activation of p38 by IL-1 occurred at a point downstream of MyD88, IRAK-1, and TRAF6, as the activation of p38 by these components was inhibited by overexpressing active Rap1AV12. Our studies therefore provide clear evidence of a role for MyD88, IRAK1, and TRAF6 in the activation of p38 by IL-1, and that the activity of these lie upstream of Ras and Rap on the pathway. Ras may therefore occupy a similar position on the p38 pathway as it does on the classical p42/p44 MAP kinase pathway, occuring upstream of the MAP kinase kinase kinase level on both pathways.

1. Palsson, E.M., et al (1999) *J.Biol.Chem.* **275,** 7818-7825

10037

LPS AND TNF$_\alpha$ INDUCE SOCS3 mRNA AND INHIBIT STAT3 ACTIVATION BY IL-6 IN MACROPHAGES

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Recent findings of several groups indicate that cytocine signaling is modulated by other simultaneously activated signal transduction pathways. Here we show that pre-incubation of human macrophages isolated from peripheral blood, rat liver macrophages or RAW 264.7 murine peritoneal macrophages with either LPS or TNF$_\alpha$ dose-dependently suppressed IL-6 induced STAT3 activation. This inhibition was not observed in primary hepatocytes isolated from rat liver or the human hepatoma cell line HepG2. Correspondingly, LPS and TNF$_\alpha$ were found to induce the expression of SOCS3 mRNA in each of the investigated type of macrophage but not in HepG2 cells. Pre-incubation of RAW 264.7 mouse macrophages with a specific inhibitor of the p38 MAP kinase (SB202190) almost completely blocks the inhibition of STAT3 activation by TNF$_\alpha$, whereas the LPS effect was only partially affected. Accordingly in RAW 264.7 and liver macrophages the TNF$_\alpha$ mediated SOCS3 mRNA expression was reduced to basic expression level by the inhibition of p38, whereas LPS-induced SOCS3 mRNA expression in RAW 264.7 mouse macrophages was only partially attenuated. We conclude from these data that the pro-inflammatory mediators TNF$_\alpha$ or LPS negatively influence IL-6 induced STAT3 activation in macrophages. This inhibition is most likely due to the induction of the de novo synthesis of SOCS3 by LPS or TNF$_\alpha$. Furthermore, evidence is given that the p38 MAP kinase might be involved, especially in the inhibitory effect of TNF$_\alpha$.

10041

Interleukin-1β (IL1β) and Tumor Necrosis Factor-α (TNFα) induce ECSIT (evolutionarily conserved signaling intermediate in Toll pathways) mRNA in hippocampal neruons

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IL1β and TNFα interact with each other to induce sleep (J. Neuroscience, 17, 5949., Am. J. Physiol., 274, R655.) Although both IL1β and TNFα upregulate each other, the common mediator by which these cytokines transduce their signals remains unknown. ECSIT acts as adapter protein that interacts with TRAF6 (TNF receptor associated factor) and regulates the processing of MEKK-1(Genes and Devel., 13, 2059) in IL-1 signaling (Curr. Opin. Immunol., 11, 13). Presently, not much is known about the regulation of ECSIT. Because of the synergy of action between IL1β and TNFα, we hypothesized that both IL1β and TNFα should upregulate ECSIT transcript if MEKK-1 mediated IL1β and TNFα signaling. Therefore, hippocampal neurons were treated with either IL1β or TNFα at 10 ng/ml in separate culture flasks. Both TNFα and IL1β upregulated ECSIT mRNA by 4-fold compared to controls. The induction of ECSIT transcripts by IL1β and TNFα suggest that MEKK-1 activation is common in the signal cascade of TNFα and IL1β. Additionally, the mechanisms of ECSIT induction by both cytokines seems to be NF-κB dependent as the inhibitors of NF-κB signaling, Zinc finger protein A20 and ABIN (J. Cell Biol.,145, 1471) were about 3-fold lower in cultures treated with IL1β or TNFα compared to controls.

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10019

Hemopoietic Growth Factors, IL-3, IL-5, GM-CSF and SLF, Induce Activation of Rac

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The Rho family of small GTPases plays a role in several cellular functions including cell cycle progression, cytoskeletal rearrangements, cell survival, chemotaxis, and phagocytosis. The activation of these GTPases is triggered by the exchange of bound GDP for GTP. Once in a GTP-bound state, these molecules undergo conformational changes resulting in recruitment and activation of downstream effectors. A pull down assay, using a GST-PAK construct, was used to measure levels of activated Rac in cell lysates. A variety of different stimuli activated Rac in hemopoietic cells. Using the mouse mast cell line, MC/9, we showed that treatment with a variety of cytokines including Interleukin-3 (IL-3), IL-5, Granulocyte/Macrophage-Colony Stimulating Factor (GM-CSF), and Steel Locus Factor (SLF) resulted in activation of Rac. Using the monocytic cell line, WEHI 274.3, we demonstrated that Colony Stimulating Factor-1 (CSF-1) also activated Rac. In the B lymphocyte cell line, WEHI-231, and LPS activated B cell blasts, cross-linking of the B cell receptor (BCR) led to activation of Rac. In contrast, stimulation of CD40 on B cells was insufficient for Rac activation. Cross-linking of CD3 on primary mouse T cells resulted in activation of Rac, where as cross-linking of CD28 did not. The kinetics of Rac activation correlated with phosphorylation/activation of p38 MAPK in all cases. The question of whether IL-4 (which activates PI-3K but not Ras/Erk or p38/JNK MAPK pathways) activates Rac will be discussed.

10039

INTERFERON STIMULATED RESPONSE ELEMENT (ISRE)- BINDING PROTEIN COMPLEXES ACTIVATED IN SINDBIS VIRUS (Hd) INFECTED CELLS

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To elucidate the host cell defense mechanisms by which the cell responds to Sindbis virus infection we have characterized cellular ISRE-binding proteins activated in the infected cell and are involved in the transcriptional induction of IFN type I inducible genes. Using electro mobility shift assays several protein complexes with an ISG15-ISRE could be detected in extracts from virus infected L929 cells which were undetectable in extracts from mock infected cells. The ISRE-binding complexes are detectable in cells infected from 9 to 20 hrs p.i. In BHK 21 and Vero cells which have a defective IFN-type I system no Sindbis virus activated ISRE-binding complexes have sofar been detected. ISRE-binding specificity, supershift experiments and conditions of formation indicate that complexes activated in L929 cells by Sindbis virus infection correspond to DRAF1/VA-IRF and ISGF3 (Daly, C. and Reich, N. Mol. Cell. Biol. 13, 3756-3764; 1993; Yoneyama,M. et al. EMBO J.98, 1087-1095; 1998). Under the experimental conditions used, transfection with Poly rI:rC induced only ISGF3. In Sindbis virus infected mouse embryo fibroblasts derived from IFN-R type I Ko mice the ISRE-binding complex comigrating with ISGF3 could not be detected. Both complexes are activated in mouse embryo fibroblasts derived from IFN-R type-II Ko mice. Viral RNA is required for activation of DRAF1. In chicken embryo fibroblasts on the other hand Sindbis virus infection activates ISRE-binding complexes with higher electrophoretic mobility and are supershifted with anti IRF-1 serum.

10032

Anti-apoptosis — Function of a Novel Protein Fragment MAJN

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Using yeast two-hybrid system, we found a molecule associated with the JAK3 N-terminal, named "MAJN", which is actually a 191 residue fragment of 1581 residue protein deduced from Gene Bank Data (D86970) with unknown function. Here, we study the function of MAJN and results show to be very interesting. Utilizing various methods including fluorescence microscopy observation, DNA fragmentation and flow cytometric analysis, we illustrated that overexpression of MAJN obviously inhibited apoptosis induced by Interleukin-2 deprival in BAF-B03β cells. To clarify the mechanism of MAJN anti-apoptosis, we analyzed several critical signalers involved in apoptosis triggered by IL-2 deprivation. Results revealed that MAJN potentially inhibited the apoptosis through blocking caspase activation in a pattern independent of Bcl-2 family members including Bax, Bcl-2, Bcl-X_L and Bcl-X_S. Moreover, overexpression of MAJN can't maintain continuous activation of Akt. Taken together, our data implied that MAJN possibly acted on the signal pathway between downstream of Akt or Bcl-2 family members and the upstream of caspase to block apoptosis induced by interleukin-2 deprivation.

10045

The expression of several pro-inflammatory genes may be regulated by mitogen activated protein kinase p38 at the level of mRNA stability.

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The regulation of mRNA stability involves AU-rich elements (AREs) within 3' untranslated regions (3' UTRs). In particular, the pentamer motif AUUUA is found in the 3' UTRs of numerous short-lived cytokine and proto-oncogene mRNAs, and overlapping repeats of this sequence have been thought to constitute mRNA instability deteminants. We have employed a tetracycline-regulated reporter system to investigate the regulation of mRNA stability by the mitogen activated protein kinase (MAPK) p38 signal transduction pathway. For example the normally stable β-globin mRNA was destabilised by the insertion of the cyclooxygenase 2 (Cox-2) 3' untranslated region (UTR), and the stability of the chimeric reporter mRNA was sensitive to activation or repression of the p38 pathway. We have subsequently found that 3' UTR fragments of several "pro-inflammatory" genes confer p38-sensitive reporter mRNA decay in this assay system. These include interferon-γ, interleukin-1β, tumour necrosis factor α and Gro-α. MAPK p38-sensitive mRNA decay is not associated with overlapping AUUUA motifs. For example a short GM-CSF 3' UTR fragment containing five overlapping pentamers confers neither instability nor p38 sensitivity. In contrast the interferon γ 3' UTR confers p38-sensitive mRNA decay, and contains only six dispersed AUUUA motifs. The tetracycline-regulated reporter system thus reveals that several genes involved in inflammation may be regulated by the p38 pathway at the level of mRNA stability. Furthermore, this system should allow us to define the determinants of sequence-specific regulation of mRNA stability by p38, and to identify RNA binding proteins which are involved in such regulation.

10031

Hsl7p, THE YEAST HOMOLOGUE OF HUMAN JAK-BINDING PROTEIN-1 (JBP1), IS A PROTEIN METHYLTRANSFERASE INVOLVED IN CONTROL OF CELL MORPHOLOGY.

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The yeast gene *HSL7* was first identified as being lethal when mutated in combination with the histone H3 gene. It was later shown to be a component of both the Ras and Cdc28 pathways. We initially identified Hsl7p as a protein of relevance to cell signaling when it was found that Hsl7p is a structural homologue of JBP1, a protein methyltransferase which binds to all Jak kinases. The requirement for Hsl7p has since been shown to be complemented by JBP1 in yeast. Yeast with a disrupted *HSL7* gene develop an elongated morphology which is largely eliminated when the cells are transformed with JBP1, but not with a construct expressing JBP1 with a mutation in the S-adenosylmethionine binding domain. Protein methyltransferase activity therefore is required for normal cell morphology. To identify the protein substrates of Hsl7p, we purified GST-Hsl7p from E. coli. Purified Hsl7p methylates histone H2A and H4 as well as human fibrillarin (GAR peptide). The immunoprecipitated Hsl7p also labels these substrates. We are now analyzing proteins that co-purify with Hsl7p in order to determine whether they are themselves methyltransferases or are regulatory factors which modify Hsl7p specificity. To verify that Hsl7p plays a role in *in vivo* protein methylation, we examined cytoplasmic proteins which become demethylated in the *hsl7Δ* yeast strain in which the *HSL7* gene is disrupted. Several proteins have been detected which require a functional *HSL7* gene to become methylated.

In summary, Hsl7p has now been conclusively proven to be a functional protein methyltransferase by several different criteria: its ability to be complemented by JBP1, its activity with enzyme preparations obtained from both yeast and *E. coli*, and the demethylation of specific proteins which occurs *in vivo* when *HSL7* is inactivated.

10033

ACTIVATION OF STAT SIGNALING IN BONE CELLS BY ESTROGEN

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Estrogen is important for the normal growth and remodeling of bone. Estrogen deficiency markedly enhances the rate of bone turnover and leads to an acceleration of age-related bone loss. Studies in the last few years have established that cytokines play a profound role in bone metabolism. In this report, we demonstrate that treatment with the gonadal hormone 17-β estradiol and resveratrol, a phytoestrogen leads to an activation of interferon regulated Stat1 protein in human osteoblast cells in culture. The estrogen receptor antagonist ICI 182, 780 blocks induction of Stat1 levels by estrogen. Stimulation of Stat1 gene expression by estrogens in osteoblastic cells suggests that components of JAK/STAT signal transduction pathway are coupled to an estrogen receptor mediated signaling pathway and may mediate distinct functional processes in skeletal system.

10025

INVOLVEMENT OF PI3-KINASE IN THE IL-1 AND TRAF-6-INDUCED NF-κB ACTIVATION

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IL-1 is a central regulator of the immune and inflammatory responses by which various inflammatory genes are induced. IL-1 signaling contains IL-1RI, IL-1RAcP, MyD88, IRAK. IRAK associates TRAF6 which activates the TAK1, NIK and IKK kinase complex with final activation of NF-κB. Other pathway of IL-1 signaling contains the PI3-kinase. However, the relationship between two pathways has not been clear. Here, we studied the role of TRAF6 and PI3-kinase to clarify the relationship of two pathways using a human glioblastoma cell line T98G, where IL-1 induced marked NF-κB as well as moderate AP-1 activation by the reporter gene assay.

IL-1 stimulation induced the recruitment of PI3-kinase to IL-1RI. LY294002, a PI3-kinase inhibitor, inhibited the recruitment of PI3-kinase and subsequent NF-κB activation. Furthermore, IL-1-induced NF-κB activation was augmented by the overexpression of wild type TRAF6 and suppressed by the transfection of dominant negative TRAF6. LY294002 had marked inhibitory effect on TRAF6-augumented IL-1 induced NF-κB activation as well. Since direct associattion of TRAF6 and PI3-kinase were observed in these conditions, these observation suggested that PI3-kinase and TRAF6 pathways are closely involved in IL-1-induced NF-κB activation.

10020

ALTERNATIVE SPLICING OF THE TGF ß - ACTIVATED KINASE 1 (TAK1)

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The protein kinase, TAK1, is involved in the signalling mechanisms of the IL1, TNF, LPS and TGFß receptors. Three splice variants of the TAK1 mRNA, designated a, b and c, have been described. We have now identified a fourth splice variant, called TAK1-d, and identified an error in the previously published TAK1-c sequence. Our data shows that the c and d variants encode proteins whose carboxyl ends differ markedly from those of variants a and b. Analysis of the human TAK1 gene sequence shows that the coding sequence is organised in 17 exons. The four splice variants result from alternative splicing of exons 12 and 16, the reading frame of exon 17 being determined by the presence or absence of exon 16. Study of the relative levels of expression of the four splice variants showed significant variations between tissues. Our evidence suggests that the alternative splicing of theTAK1 mRNA may have important functional implications.

10048

Common γ chain-mediated activation of JAK3 is not required for the induction of CXCR4 expression on *in vitro* generated CD4+ CCR7+ central memory T cells.

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CXCR4 is a chemokine receptor which together with its ligand, stromal factor-derived factor 1 (SDF-1), is involved in the regulation of leukocyte migration and other immunologic processes. In addition, CXCR4 has been identified as a co-receptor for HIV-1 entry. We have previously shown that interleukin (IL)-2, IL-4, IL-7 and IL-15 are able to induce the expression of functional CXCR4 at the surface of *in vitro* generated CD4+ CXCR4- CCR7+ central memory T cells, resulting in enhanced SDF-1-mediated *in vitro* migration and enhanced susceptibility to infection with an X4 HIV-1 strain. Since the receptors for these cytokines share the common γ (cγ) chain, these results suggest that JAK-3, a kinase specifically involved in cγ-mediated signaling, may play a role in the induction of CXCR4 expression. Indeed, selective inhibition of JAK3 in central memory T cells by the inhibitor WHI-P131 blocked IL-2- and IL-15-mediated induction of CXCR4 expression. In contrast, the IL-4- and IL-7-mediated induction of CXCR4 expression was not affected by WHI-P131, even though proliferation, induced by these cytokines, was totally abrogated. As the mitogen-activated protein kinase (MAPK) pathway regulates cell proliferation as well as differentiation, we assessed whether MAPK activation was differentially activated in IL-2/IL-15- versus IL-4/IL-7-stimulated T cells, cultured in the absence or presence of WHI-P131. Both IL-2 and IL-15, but neither IL-4 nor IL-7, activated the ERK-1 and ERK-2 MAPKs in these cells. Thus, the inhibitory effect of WHI-P131 on CXCR4 expression was observed only in cells activated by cytokines (IL-2/IL-15) which recruit the MAPK pathway. In this regard, it is of note that treatment with WHI-P131 augmented IL-2/IL-15-mediated MAPK activation. Taken together, these results show that cγ chain-mediated activation of JAK3 is not required for the induction of CXCR4 expression and, moreover, that increased MAPK activation may inhibit induction of CXCR4 expression.

10047

Induction of Ref-1 Ensures AP-1 Activation in Oxidative Intracellular Environment of IL-2-stimulated Cells

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The redox regulation of cellular signaling has multiple functions in cell physiology. In this study, we investigated the potential redox regulation involved in interleukin-2 (IL-2) signaling using IL-2-dependent BA/F3beta cells. IL-2-induced DNA binding and transcriptional activity of activator protein-1 (AP-1) were shown sensitive to oxidation analyzed by electromobility shift assays (EMSA) and reporter gene assays, respectively. However, flow cytometric assay by using 2',7'-dichlorofluorescin diacetate as redox probe indicates that IL-2 stimulation produces an oxidative intracellular environment, which seemed to conflict with the requirement of AP-1 activation. On the other hand, Western blot analysis shows that IL-2 upregulates the expression of redox factor-1 (Ref-1). EMSA of nuclear extracts immunodepleted of Ref-1 protein demonstrated that the IL-2-induced AP-1 DNA binding is dependent on the presence of Ref-1. This was confirmed by the restoration of AP-1 DNA binding upon the re-addition of immunoprecipitated Ref-1 to the immunodepleted extracts. Similarly, AP-1 transcriptional activity was enhanced by the overexpression of Ref-1 and attenuated by the introduction of anti-Ref-1. These results suggest that the induction of Ref-1 ensures AP-1 activation in the oxidative intracellular environment following IL-2 stimulation.

10046

DYSREGULATED HEMATOPOIESIS IN MICE LACKING THE ADAPTOR PROTEIN LNK.

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Lnk is a 70 kDa adaptor protein expressed mainly in hematopoietic cells. It contains a proline rich region, a pleckstrin homology (PH) domain, an SH2 domain and numerous potential phosphorylation sites. This protein forms a new subfamily of SH2 proteins together with APS and SH2B, which have been implicated in the regulation of growth factors and cytokines signalling.
To elucidate the physiological function of Lnk, we generated mice bearing a targeted disruption of the *Lnk* locus. Lnk-deficient mice were viable, fertile and normal in appearance. However, consistent with its expression in the hematopoietic compartment, Lnk-deficient mice displayed marked changes in their hematopoietic profiles, including bone marrow fibrosis and splenomegaly. Histological analysis together with flow cytometry and colony assays indicated an increased in the myeloid, erythroid and megakaryocyte lineages. Peripheral blood analysis showed a marked elevation in the white blood cell counts and platelet levels of homozygous mice compared to wild type. For all the parameters studied, the Lnk heterozygous mice displayed an intermediate phenotype indicating a dosage effect.All the hematopoietic defects present in the Lnk-deficient mice resemble the human myeloproliferative disorders, characterized by erythrocytosis, granulocytic and megakaryocitic hyperplasia and myelofibrosis. Therefore, our data suggests an important role for Lnk in hematopoiesis and in controlling proliferation and signalling by the cytokine receptor family.

10015

INTERFERON-α AND β, PROTECT FROM APOPTOSIS, AND INDUCE GROWTH IN PRIMARY B-LYMPHOCYTES, THROUGH A PI3-KINASE DEPENDENT PATHWAY.

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Interferon-α and β, when added to human primary B-lymphocytes, protected the cells from undergoing apoptosis. The PI3-kinase inhibitor Ly294002 totally abolished the protecting action of interferon. In submitogenic doses of anti-IgM antibodies, interferon-α and β (IFN) stimulated growth of human primary B-lymphocytes in a dose dependent way. The co-stimulatory activity is as potent as that of interleukin 2 or interleukin 4, and could be shown to involve an increased sensitivity to the mitogenic anti-IgM antibody. We found an increased DNA-synthesis and a down regulation of cyclin-dependent kinase inhibitor p27kip1, when IFN was added to the anti-IgM preactivated cultures after 2 days. The PI-3 kinase inhibitor Ly 294002 inhibited the increased DNA-synthesis and down regulation of p27. In search for molecular mechanisms we found that the PI3-kinase downstream target Akt (PKB) was phosphorylated on Ser-473 in response to IFN in both unactivated and anti-IgM preactivated cells. No phosphorylation of Akt was detected in the presence of the inhibitor, Ly294002. We conclude that IFN is a co-stimulator of human primary B-cells and induces both growth and blocks apoptosis via signalling through PI3-kinase and propose that the serine/threonine kinase Akt is the key mediator of the growth stimulatory and anti-apoptotic effects induced by IFN.

10011

FORCED IFN-γR2 CHAIN EXPRESSION INDUCES IFN-γ MEDIATED APOPTOSIS IN HUMAN MALIGNANT T CELLS

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The heterodimeric IFN-γR is formed of two chains. We investigated the expression of both IFN-γR chains on human T, B and myeloid cells. The binding chain (IFN-γR1) is highly expressed on the membrane of malignant T, B and myeloid cells. Conversely, the transducing chain (IFN-γR2) is highly expressed on the surface of myeloid cells, moderately expressed on B cells and poorly expressed on the surface of T cells. The differential expression of the IFN-γR2 chain changes the cell response to IFN-γ. It promotes the growth of human T cells while it inhibits that of B and myeloid cells. In effect, in IFN-γR2 transfected Jurkat T cells with up-modulated surface expression of IFN-γR2 chain, addition of IFN-γ increases their apoptosis. Binding of IFN-γ to T cells with a low expression of the IFN-γR2 chain induces low levels of IRF-1, Fas and Fas-L and no caspase-1 and caspase-3 expression, and promotes their growth, whereas its binding to those with forced IFN-γR2 chain expression induces high levels of IRF-1, Fas, Fas-L, caspase-1 and caspase-3 expression and promotes their apoptosis. These data indicate that IFN-γ-mediated apoptosis on T cells is strictly dependent on the extent of IFN-γR2 chain surface expression.

10014

StIP: a WD Repeat Stat Interacting Protein

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IL-6 is a pleiotropic cytokine that plays an important role in hematopoiesis and the "acute phase response" to both infectious and inflammatory stimuli. IL-6 mediates these responses through the activation of Stat3. In an effort to identify regulatory proteins that interact with Stat3, a yeast two hybrid screen was carried out using a portion of Stat3 that includes the conserved "coiled coil" domain. This lead to the isolation of a ubiquitously expressed 92 kDa Stat Interacting Protein (StIP). StIP has an open reading frame of 831 amino acids and other than 12 WD40 repeats, does not have any other recognizable functional motifs. WD repeat proteins are involved in a wide variety of cellular functions including transcriptional regulation, chromatin remodeling, cytoskeletal assembly, mitotic-spindle formation, vesicular trafficking, and protein degradation. The WD repeat domain in all of these proteins functions to coordinate the sequential and/or simultaneous interactions involving several sets of proteins. Biochemical studies confirmed the ability of StIP to bind unphosphorylated Stat3 as well as several Jaks. Based on these observations, and the ability of WD40 domains to coordinate protein- protein interactions, further studies have been designed to test the model that StIP is a scaffolding protein in cytokine signal transduction. In support of this hypothesis, we have been able to show that a dominant negative mutant of StIP blocks Il-6 mediated Stat3 activation, nuclear translocation, and reporter gene expression. These studies indicate that StIP regulates the ligand dependent activation of Stat3 and may serve as a scaffolding protein, potentiating the interaction between components of the JAK STAT signaling pathway.

10009

CHARACTERIZATION OF THE INTERACTION REGIONS OF JAK1 AND GP130 BY MUTATIONAL ANALYSIS.

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Gp130, the common signal transducing receptor subunit of the Interleukin-6 (IL-6) type cytokines, associates with the Janus kinases Jak1, Jak2 and Tyk2, of which Jak1 was shown to be essential for signal transduction. In order to study the requirements for gp130-Jak interaction and signal propagation, we performed a mutational analysis of the N-terminal part of Jak1 and of the membrane-proximal region of gp130.
Using the fold-recognition approach, a ubiquitin-like β-grasp domain was identified within the N-terminal region of Jak1. Several point mutations introduced into a putative loop region abrogated gp130 association in transient COS7 transfectants and IL-6-dependent STAT activation. Substitution of Y107 also resulted in loss of receptor binding. This amino acid residue corresponds to Y100 of Jak3 which was previously found to be exchanged in a SCID patient. The characteristics of these Jak1 mutants will be discussed in the context of the recently solved structure of a FERM domain (of moesin) which is predicted to be present also in Janus kinases.
In gp130, a single amino acid substitution ($W_{666}A$) in the membrane-proximal interbox1/2 region abrogated activation of STAT transcription factors and the proliferative response of pro-B cell transfectants. Moreover, association of Janus kinase Jak1 was prevented. No signalling of heterodimeric IL-5R/gp130 chimeras occured in COS-7 cells even when only a single cytoplasmic chain of a gp130 dimer contained the $W_{666}A$ mutation, indicating that it acts dominantly.

10008

Cytokine response gene 8 (CR8) regulates the G1-S phase transition and cellular survival

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Interleukin-2 plays a pivotal role in directing the proliferation, survival and differentiation of T lymphocytes. To elucidate how IL-2 promotes such diverse cellular responses, we have utilized a sulfhydryl-labeling and affinity purification (SLAP) method to identify IL-2-induced immediate-early genes from human T cells (PNAS 90:2719, 1993). Eight cytokine-responsive (CR) genes were isolated by differential hybridization of an IL-2- and cycloheximide-treated T cell cDNA library. One of these genes, CR8, encodes a 45 kDa basic helix-loop-helix (bHLH) protein. Ectopic CR8 expression slows the G1-S phase cell cycle transition, and also inhibits the apoptotic response. These results indicate that IL-2 signals diverse T cell functions through the induction of target genes, and demonstrate that the characterization of CR genes will provide new insights into the understanding of immune system modulation by cytokines.

10007

IN VIVO REGULATION OF THE dsRNA-DEPENDENT PROTEIN KINASE PKR BY THE CELLULAR GLYCOPROTEIN p67

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Regulation of eIF2α phosphorylation is critical to the maintenance of cellular homeostasis and the kinases that regulate eIF2α phosphorylation are subject to complex and multidimensional controls. A cellular 67 kDa glycoprotein (p67) has been proposed to have an important role in eIF2α kinase activity. In order to determine the *in vivo* regulatory interaction between p67 and PKR we have used a vaccinia virus (VV) expression system that successfully mimics PKR control pathways. Recombinant VV strains were constructed that constitutively expressed p67 and inducibly expressed PKR. The stable expression of p67 reduced the PKR mediated anti-viral response and apoptosis. This inhibition was correlated with decreased eIF2α phosphorylation in the presence of activated PKR. In addition, PKR mediated inhibition of reporter gene expression and general protein synthesis were abrogated by p67 expression. Significantly, PKR associated regulation of NF-κB transcriptional activity was unaffected by p67. These data demonstrate that *in vivo* p67 effectively and specifically regulates PKR translational effects and suggest that it may be useful in the further dissection of diverse PKR mediated pathways.

10022

Identification of Novel Components of Jak-Mediated Signalling Pathways.

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There is persuasive evidence that JAKs have additional roles in cytokine signalling besides phosphorylation of receptors and STATs. Our objective is to identify 'novel' signalling molecules that have not previously been implicated in JAK-mediated signalling. Phosphotyrosine profiles of whole cell extracts, or subcellular fractions, of unstimulated and cytokine-treated cell lines have been analysed and confirm the existence of ligand-inducible phosphotyrosine species not corresponding to known participants in JAK-STAT signalling. Recombinant SH2 domains, cytokine receptor motif peptides, anti-phosphotyrosine antibodies and 2D phosphotyrosine profiling are being used to select molecules that are tyrosine phosphorylated in response to cytokine. Stable transfectants of chimaeric receptor constructs that retain the ability to bind JAKs but lack STAT-recruitment sites are also being employed to specifically identify JAK-dependent, STAT-independent signals. Novel tyrosine phosphorylated proteins activated by signalling through the chimaeric receptors, or exhibiting cytokine specificity or differential profiles in cell lines mutated in individual JAKs, will be purified and identified by mass spectrometry.

10021

ANALYSIS OF GP130, LEUKEMIA INHIBITORY FACTOR RECEPTOR AND ONCOSTATIN M RECEPTOR REVEALS SHARED AND DISTINCT SIGNALLING PROPERTIES

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The IL-6-type cytokines IL-6, IL-11, LIF, OSM, CNTF, CT-1 and the novel member NNT-1/BSF-3 transduce their signals via activating receptor complexes which either lead to a homodimerization of the common receptor subunit gp130 or a heterodimerization of gp130 with either LIFR or OSMR. Thereby, intracellular signalling via Janus kinases (Jaks) and STAT transcription factors is initiated. Using a chimeric receptor system we analyzed the contributions of LIFR and OSMR to signal transduction in the context of a heterodimer with gp130. LIFR as well as the OSMR can bind Janus kinases (Jak1, Jak2 and Tyk2 for LIFR, Jak1 and Jak2 for OSMR). Thereby they provide, in combination with gp130, the second kinase necessary for activation of the receptor complex. Only one receptor chain within a gp130/LIFR or gp130/OSMR heterodimer has to contain the membrane-distal part harbouring STAT-recruitment sites. We were also able to show for the first time in a comparative study that the signal transducing receptor chains differ in their recruitment pattern of proteins. While gp130 recruits the tyrosine phosphatase SHP-2 via its tyrosine residue 759, the OSMR binds Shc p52 and p66 isoforms. Mutational analysis of the OSMR revealed the binding site for Shc proteins in this receptor subunit. We further demonstrate that the Shc recruitment is necessary for activation of the MAP kinases Erk1/Erk2. As Shc proteins are involved in many cellular signalling pathways, the observation that OSM activates these proteins is an important step in elucidating distinct signalling properties exerted by this cytokine.

10012

IDENTIFICATION OF SUBLINES OF U937 CELLS EXHIBITING DIFFERENT LEVELS OF TNF RECEPTORS AND DISTINCT PATTERNS OF RECEPTOR-MEDIATED RESPONSES TO TNF

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Two lines of U937 cells (human histiocytic lymphoma), termed $U937_M$ and $U937_G$, have been identified according to differences in sensitivity to tumor necrosis factor (TNF). The $U937_M$ cell line revealed sensitivity to cytotoxic action of TNF in the presence or absence of protein synthesis blocker, cycloheximide (CHX). The $U937_G$ cells did not exhibit sensitivity to TNF in the absence of CHX, which was regained however in its presence. EMSA assays of nuclear extracts of cells treated with TNF in the absence of CHX and cytotoxicity tests performed in the presence of different concentrations of protease inhibitors (TPCK and TLCK) suggested an NF-κB-dependent mechanism resulting in protection of $U937_G$ cells from cytotoxic action of TNF. However, analysis performed with PSI, the more specific inhibitor of NF-kB activation showed that though this transcription factor is important for survival of U937 cells in culture, it most probably does not affect sensitivity of the cells to TNF. Different expression levels of TNF receptors (TNFR55 and TNFR75) on the cell surface of both U937 cell lines may also suggest differences in their engagement in the induction of cytotoxic reaction on one hand and in the initiation of protection mechanisms on the other. In fact, cytocidal activity of TNF could be fully blocked by anti-p55 but not by anti-p75 antibodies in the $U937_G$ cells, while in the $U937_M$ cells both types of antibodies were able to block partially the cytocidal response to TNF. Similar pattern of TNF inhibition was observed also in the case of TNF-induced activation of NF-κB transcription factor. Cytogenetic and fingerprinting analysis of the U937 sublines, their subclones as well as of the freshly obtained ATCC U937 cells confirmed their common origin. Interestingly, the freshly obtained ATCC U937 cells turned to be resistant to TNF in the absence of CHX, presented also similar pattern of TNF receptor expression as the TNF-resistant $U937_G$ subline. This observation underscores variability of the U937 cell line, which is used widely as a typical model of TNF-induced apoptosis and is described by most authors as a TNF-sensitive cell line.

10042

PROTEIN KINASE R (PKR) IN RAT ISLETS OF LANGERHANS AND RIN-CELLS IS UPREGULATED BY CYTOKINES

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PKR is a 62 kDa serine-threonine kinase related to antiviral and apoptotic pathways in most cells where it deactivates eIF-2α through phosphorylation and thereby inhibits protein synthesis. The antiviral effect involves binding ds-RNA, whereby PKR becomes autophosphorylated. It has been suggested that PKR is central in the IFN-induced enzymatic pathways leading to apoptosis of infected cells. Since ds-RNA in previous studies have been shown to cause β-cell apoptosis, the aim of this study was to demonstrate the existence of PKR in rat islets and RIN-cells by Western blotting and protein kinase assays. PKR was constitutively expressed in these cells, and stimulation with IL-1 or IFN-γ caused a 30-40 % increase in PKR protein-content. TNF-α was not able to mediate upregulation in RIN-cells, although TNF-α activates PKR in other cells. In conclusion, PKR may participate in signalling in cytokine mediated β-cell apoptosis.

10030

Dexamethasone destabilises cyclooxygenase-2 mRNA by inhibiting mitogen activated protein kinase p38.

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Glucocorticoids have been shown to destabilise cyclooxygenase-2 (Cox-2) mRNA by a mechanism involving shortening of the poly(A) tail. In contrast, activation of the (MAPK) p38 signal transduction pathway results in the stabilisation of Cox-2 mRNA. We have used a tetracycline-regulated reporter system to investigate mechanisms of regulation of Cox-2 mRNA stability by the synthetic glucocorticoid dexamethasone (dex), and report that dex regulates Cox-2 mRNA stability by inhibiting MAPK p38. Reporter mRNA stability was sensitive to dex only in the presence of factors or treatments which stimulated p38. For example stabilisation by MKK6 (an activator of p38) was dex-sensitive, whilst stabilisation by MAPKAPK-2 (an effector lying downstream of p38) was dex-insensitive. Dex and SB203580 (an inhibitor of p38) exerted very similar effects upon mRNA stability, with no additive or synergistic action. Finally, the regulation of mRNA stability by dex or by p38 was mediated by precisely the same short region of the Cox-2 3' UTR. We show that p38 activity is negatively regulated by dex in HeLa cells stimulated with ultraviolet light or IL-1, and in RAW264.7 mouse macrophage cells stimulated with lipopolysaccharide. The MAPK p38 pathway controls the expression of several genes at the level of mRNA stability. The findings described here suggest that glucocorticoids may negatively regulate gene expression by means of p38 inhibition, and consequent mRNA destabilisation. This represents an important novel point of convergence of two signal transduction pathways which profoundly influence pro-inflammatory gene expression.

10016

STAT1 AS A REGULATOR OF CONSTITUTIVE GENE EXPRESSION

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Tyrosine-phosphorylated Stat1 homodimer is the major regulatory factor in the signaling pathway mediated by interferon-gamma. However, non-tyrosine phosphorylated Stat1 can also regulate constitutive gene expression. Transcription of the low molecular mass polypeptide (LMP) 2 gene, which requires Stat1, is also supported by Stat1 Y701F, which does not form dimers involving SH2-phosphotyrosine interactions. A dimer of recombinant unphosphorylated Stat1, formed through the interaction of the N-terminal domains of two monomers of Stat1, can bind to the ICS-2/GAS element of the LMP2 promoter. Stat1 can also interact with IRF1 and, *in vivo*, the ICS-2/GAS element of the LMP2 promoter is constitutively occupied by a complex containing both non-tyrosine phosphorylated Stat1 and IRF1. Stat1 Y701F does not activate the IRF1 gene, which also contains a GAS site but not an adjacent ICS-2 site. By analogy, Stat1 may regulate the constitutive expression of other genes whose promoters bind to complexes of unphosphorylated Stat1 with IRF1 or other transcription factors.

10028

Mitogen-activated protein (MAP)s kinase and NFkB regulate production of Tumor necrosis factor-α in mast cells after Substance P stimulation.

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We have investigated in mast cell the regulation of the production of intracellular TNF-α after stimulation with Substance P (SP). Lysates of purified peritoneal mast cell were incubated with phospho-specific pAb versus MAP kinases: p42/44, p38 and JNK and we obtained evidences that p42/44 and p38 are activated after SP-stimulation. Selective inhibition of p42/44 and p38 by PD98059 and SB203580 indicated that these kinases contributed to the production of TNF-α after SP-stimulation. Intracellular production of TNF-α was evaluated as protein and mRNA by Western blot and quantitative RT-PCR. The inhibitors, PD98059 and SB203580 alone, do not interfere with TNF-α protein synthesis or TNF-α mRNA levels. By using PD98059 (50μm) and SB203580 (20μm), before SP-stimulation the signalling pathway for SP was blocked and the TNF-α mRNA and protein enhancement was reduced. The transcription factor NFkB, that is inhibited by (E)-4-Hydroxynonenal (HNE), is involved in the production of TNF-α mRNA both as basal level and as enhancement after SP-stimulation. In fact, 15min. after the treatment with HNE, the mRNA and the TNF-α protein level showed lower values than the control. These results suggest that TNF-α expression in mast cell after SP-stimulation is probably regulated by p42/44 and p38 mainly post-transcriptionally and by NFkB at the transcriptional level.
In conclusion we suggest that: i) ERK, p38 and NFkB pathways, all activated upon stimulation with SP, are critical for and cooperatively contribute to the induction of TNF-α expression in mast cells ii) these three pathways not all act on the gene and therefore appear to be not redundant iii) selective inhibition at least of 2/3 signalling pathways is necessary for blocking the production of TNF-α.

10017

Inhibition of MITF promoter activity, in melanoma F10.9 cells, reveals regulation by IL6RIL6 of the Pax3, beta-catenin, and CRE signaling pathways.

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The HLH-LZ transcription factor MITF-M is a marker of the melanocytic lineage, and regulates genes important in melanogenesis as tyrosinase (Tyr) and tyrosinase related protein-1 (TRP-1). In F10.9 melanoma, stimulation of the gp130 transmembrane signaling molecule with a fusion protein of interleukin-6 receptor and interleukin-6 (IL6RIL6) leads to extinction of melanogenesis. We established that the Tyr activity and mRNAs are down regulated to undetectable levels, as a result of inhibition of MITF transcription. Luciferase (Luc) reporter plasmids containing from 2kb to 400bp of the human MITF gene upstream sequence were transfected in F10.9 cells. Luc activity was decreased 5-10 folds by IL6RIL6 in 48 hours assays. The 400bp sequence contains a complex array of potential regulatory sites. Three of them were shown in other laboratories to bind positive regulators of MITF transcription, namely, from 5' to 3', the paired homeodomain gene Pax3, the TCF/LEF-1 factors, and the c-AMP responsive element binding protein (CREBP). In our cell system, inactivating mutations of Pax3 or CRE sites, or internal deletion of the LEF-1 site, reduced respectively by 5-6 folds, or by 2-folds, the constitutive activity of the 400bp construct. Each mutation partially diminishes the fold reduction by IL6RIL6. We show that the expression of Pax3 mRNAs and protein, and the one of beta-catenin protein (essential in TCF-/LEF-1 factors activity) are reduced early (6 hours) in IL6RIL6 treated samples. Moreover, transcription activity of a CRE-TK-Luc vector is reduced. Though we cannot exclude that other IL6RIL6-regulated sites exist on the MITF promoter, this study reveals that three signaling pathways contributing to MITF gene transcription are regulated by IL6RIL6.

10018

MECHANISM OF INTERLEUKIN-1 CYTOTOXICITY IN HeLa CELLS

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Interleukin-1 (IL-1) is a pleiotropic cytokine playing major role in the regulation of inflammatory and immune responses. It also takes part in the regulation of growth, differentiation and many metabolic processes. IL-1 may exert cytocidal activity in a limited number of cell types, usually in the presence of metabolic inhibitors. The mechanism of IL1-induced cell death remains unknown. The aim of this study was to analyze the cytocidal effect mediated by IL-1 in the presence of the translation inhibitor cycloheximide (CHX) in HeLa cells. We found that the cytotoxic action of interleukin-1 reveals significant similarities to apoptosis induced in the same conditions by tumor necrosis factor (TNF). It shows similar cell cycle and DNA degradation pattern, and can be blocked by caspase inhibitor Z-VAD.fmk. Following application of IL-1 we found TNF-like activity in supernatants and substantial increase of TNF in the cytoplasm of HeLa cells which was confirmed by antibody blocking experiments and by specific ELISA. Thus, the cytocidal effect of IL-1 in HeLa cells may be dependent on release of small amounts of TNF. This phenomenon may contribute also to cooperation between the two cytokines observed in several biological activities, including cytocidal effect induced upon TNF-sensitive cells.

10027

SIGNAL TRANSDUCTION MEDIATED BY CD4, AN HIV AND INTERLEUKIN-16 RECEPTOR, ON MONOCYTIC CELLS.

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The CD4 molecule is a 56 kDa membrane glycoprotein expressed by a subset of T cells, by cells of the monocyte/macrophage lineage, and by eosinophils and dendritic cells. CD4 serves as a coreceptor for HIV infection. In T cells, CD4 mediates signal transduction by virtue of its association with the protein tyrosine kinase $p56^{lck}$; this association does not exist in monocytes. It was our goal, therefore, to elucidate the mechanism(s) of monocyte CD4 signal transduction. We have shown that stimulation of Thp-1 monocytic cells with antibody to CD4 results in a calcium flux, as well as in the time-dependent tyrosine phosphorylation of various proteins having molecular weights of approximately 180, 140, 120, 110, 85, 65, 55, 50 and 35 kDa. We have identified the 140 and 85 kDa proteins as phospholipase C -gamma (PLC-γ), and the regulatory subunit of phosphatidylinositol-3 kinase (PI3-K), respectively. Using immunoprecipitation western and immunoblotting, we have been unable, however, to show any direct association between CD4 and PLC-γ, PI3-K, or other signalling proteins. In an attempt to identify proteins capable of associating with CD4's cytoplasmic tail, we generated a GST-$CD4_{cyt}$ fusion protein which we used in Far western blots and immunoprecipitation experiments. In both types of experiments, the GST-$CD4_{cyt}$ fusion protein routinely associated with 45 and 55 kDa proteins.

10026

MODULATION OF IFN-INDUCED RESPONSES BY INHIBITORY EFFECTS OF GM-CSF ON STAT-1 ACTIVATION.

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We have investigated the effects of GM-CSF on biological signals induced by IFN's in hematopoietic cell lines and in primary human hematopoietic stem cells. In U937 cells, EMSA revealed that GM-CSF significantly inhibited IFN-induced STAT-1 activation. This inhibition is caused by downregulation of STAT-1 tyrosine phosphorylation. Thus, in U937 cells IFN-induced expression of IRF-1 and PKR was completely abolished at mRNA and protein levels. To evaluate the biological significance of GM-CSF-induced inhibition of IRF-1 expression, we monitored IRF-1-dependent induction of MHC-I or TAP genes. In U937 cells, constitutive and IFN-induced MHC-I and TAP-1 expression was suppressed by GM-CSF.
Further, we examined modulation of IFN-induced responses by GM-CSF in primary human $CD34^+$ cells ex vivo. Again, GM-CSF showed strong inhibitory effects on IFN-α induced IRF-1 mRNA levels and on IFN induced MHC-I expression. Further evidence that this mechanism is operational in primary human hematopoetic cells in vivo was obtained by administration of GM-CSF to cancer patients, which also resulted in downregulation of IRF-1 mRNA levels in primary mononuclear cells.
In conclusion, our data provide evidence that in a physiological setting, where primary human hematopoietic cells are exposed to a multitude of cytokines simultanously, STAT-1 phosphorylation plays an important role in modulating antagonistic signals.

10043

MOLECULAR CHARACTERIZATION OF SIGNALING PATHWAYS LEADING TO IRF-3 ACTIVATION

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Infection of host cells by viruses results in to the activation of multiple signaling pathways leading to the transcriptional activation of host genes involved in the response to virus infection. Among the transcription factors mediating the immediate response to virus infection is interferon regulatory factor 3 (IRF-3) which is activated by phosphorylation of serine and threonine residues in the C-terminal region. In the course of identifying signaling pathways implicated in the activation of IRF-3, Western blot analysis revealed that IRF-3 is a phosphoprotein that exists as two forms in several uninfected cell types. The first form (I) represents nonphosphorylated IRF-3, whereas the second form (II) is sensitive to phosphatase treatment and reveals basal phosphorylation of IRF-3 in a region distinct from the C-terminal domain. Following virus infection, two additional slowly migrating forms of IRF-3 are detected, III and IV, that are sensitive to phosphatase treatment and represent C-terminal phosphorylation of IRF-3. We present convincing data showing that only forms III and IV are present in the nucleus of virus infected cells, and that only those forms have transcriptional activity as reflected by DNA binding capacity and CBP coactivator association. Interestingly, treatment of cells with DNA damaging agents and stress inducers also induces IRF-3 phosphorylation as observed by the accumulation of form II. However, no nuclear translocation or transcriptional activity of IRF-3 can be detected in different target cells treated with these phamacological agents. The N-terminal region of IRF-3 is necessary for stress inducers and DNA damaging agents-induced phosphorylation of IRF-3 and studies are underway to identify the role of N-terminal phosphorylation in the regulation of IRF-3 activity.

10044

T-TYPE CALCIUM CHANNEL INHIBITION DOES NOT INFLUENCE CYTOKINE-INDUCED ACTIVITY OF MITOGEN-ACTIVATED PROTEIN KINASES IN PANCREATIC β-CELLS

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The cytokines interleukin (IL)-1β, tumor necrosis factor (TNF)-α and interferon (IFN)-γ are cytotoxic to pancreatic β-cells. When exposed to β-cells these cytokines activate the mitogen-activated protein (MAP) kinases c-jun N-terminal kinase (JNK), p38 and extracellular signal-regulated kinases 1/2 (ERK1/2). However, cytokine-induced signal transduction in β-cells also involves signalling through T-type calcium channels. Both inhibition of JNK or T-type calcium channels have individually been shown to prevent cytokine-induced β-cell apoptosis. In this study we investigated if blocking T-type calcium channels influence the cytokine-induced increase in MAP kinase activity determined by phosphotransferase assay in mouse β-TC3 cells. After 1 h exposure to a mixture of cytokines (25 U/ml IL-1β, 100 U/ml TNFα, 100 U/ml IFNγ) an increase in activity of JNK, p38 and ERK1/2 was found. Blocking T-type calcium channels with either $NiCl_2$ (10 μM) or mibefradil (1 μM) did not influence the cytokine-induced increase in MAP kinase activity (n=7). Similarly, after 6 h exposure to cytokines the observed increase in MAP kinase activity was not influenced by $NiCl_2$ or mibefradil (n=5). These findings were reproduced using the rat insulinoma (RIN) 5-AH-T2B cell line. These data provide evidence that the cytokine-induced signalling through T-type calcium channels is not involved in regulating the cytokine-induced increase in MAP kinase activity. More likely, the calcium-signalling through T-type channels is a down-stream event of the MAP-kinase cascade.

10024

Transactivation by the p65 subunit of NFκB in response to IL1 involves MyD88, IRAK1, Traf6 and Rac1

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Nuclear factor-κB is a key pro-inflammatory transcription factor which is activated on stimulation with the cytokine interleukin-1 (IL-1). We have previously shown that, in addition to the well-characterised pathway leading to IκB degradation, IL1 also regulates a separate pathway mediating the transactivating activity of the p65 subunit of NFκB which involves the low molecular weight G protein Rac1. We have examined the involvement of the known components of the IL1 signalling pathway – MyD88, IRAK1 and 2, and Traf6 – in this pathway using the murine thymoma cell line EL4.NOB-1. Transient transfection of cells with plasmids encoding wild-type MyD88, IRAK1 and Traf6 drove p65-mediated transactivation whereas IRAK2 alone was unable to drive this response. Dominant negative forms of IRAK1 and Traf6 completely inhibited the IL1-induced response, whereas transient transfection of cells with MyD88 mutants resulted in 30-50% inhibition of the response. Together these results indicate that MyD88, IRAK1 and Traf6 are important downstream regulators of IL1-mediated p65 transactivation. A dominant negative mutant of Rac1, RacN17, inhibited wild-type MyD88, IRAK1 and Traf6 induced transactivation, thus placing Rac1 downstream of these proteins on this pathway. In addition, constitutively active RacV12-mediated transactivation was not inhibited by dominant negative MyD88 or Traf6, confirming that Rac1 lies downstream of MyD88 and Traf6 on this pathway. Preliminary results indicate that Rac1 associates in a complex with both MyD88 and the IL1 receptor accessory protein, confirming the importance of Rac1 in IL1 signal transduction.

10023

THE HUMAN JAK-BINDING PROTEIN-1 (JBP1): ISOLATION AND CHARACTERIZATION OF ITS PROTEIN METHYLTRANSFERASE ACTIVITY.

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To investigate the mechanisms of interferon signaling, a yeast two-hybrid screen was used to identify proteins which interact with Janus kinase-2 (Jak2). A number of Jak-binding protein cDNAs have been isolated, including the JBP1 cDNA. JBP1 is homologous to several other protein methyltransferases. Homologues of JBP1 have been found in *S. pombe*, *C. elegans* and S. cerevisiae. Further studies indicated that JBP1 also binds to Jak1, Jak3 and Tyk2, indicating that JBP1 plays a significant role in cytokine signaling. Early on the function of JBP1 was suggested by its homology to other protein methyltransferases and by the fact that JBP1 could methylate several proteins *in vivo*. Purification of GST-JBP1 from *E. coli* has further enabled us to investigate the substrate specificities of this enzyme *in vitro*. Affinity-purified GST-JBP1 and JBP1 immunoprecipitated from HeLa cells actively methylated histone H2A and H4. However, myelin basic protein, which is methylated by immunoprecipitated JBP1 preparations, could not be efficiently methylated by GST-JBP1. JBP1 is therefore an active protein methyltransferase, but another factor, present in the immunoprecipitate, is likely to be required to methylate certain substrates efficiently.

Clinical use of cytokines and interferons

11005

In vivo ANTIVIRAL REACTIVITY IN CHRONIC HIV INFECTION

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The Human Immunodeficiency Virus (HIV) usually produces a chronic, asymptomatic infection. Treatment with Highly Active Anti-Retroviral Therapy (HAART) is effective in suppressing viral replication but cannot cure the infection: interruption of therapy results in a rapid relapse of plasma virus. Acute viral infections are combated and controlled by the host antiviral defenses, which are attributable to both innate and acquired immune systems. Most of the acquired antiviral reactivity in experimental models, such as LCMV, is mediated by CD8+ T cells, which depend upon "help" in the form of interleukin 2 (IL2) and other cytokines derived from CD4+ T cells. To determine whether individuals infected chronically with HIV can mount a significant antiviral response, subjects who had recovered normal lymphocyte counts on HAART + daily low-dose IL2 therapy have volunteered to discontinue HAART but to continue to receive IL2. The antiviral host response was then monitored by frequent viral and lymphocyte subset determinations thereafter. Thus far, all subjects have undergone a viral relapse, with plasma HIV RNA becoming detectable within 19 +/- 3 days. Subsequently, the HIV concentration doubled every 1.6 +/- 0.3 days, reaching a peak 2.5 weeks later. Thereafter, there was a "spontaneous" decline of plasma HIV RNA, with levels decreasing by a mean of 10-fold to reach a trough within the next 2 weeks. Coincident with the rise and fall of the plasma virus was a marked increase in circulating CD8+ T cells to a mean of ~ 2-fold greater than the baseline concentration. By comparison, circulating CD4+ T cells decreased by only 24% transiently at the peak of the viremia, while NK cells remained unchanged. Some individuals have undergone a second interruption of HAART, and the peak and trough viral concentrations have been attenuated > 10-fold compared with the 1st interruption. Thus, individuals infected chronically with HIV have readily detectable antiviral reactivity, and monitoring viral and lymphocyte dynamics after interruption of antiviral therapy provides discrete endpoints for future clinical trials.

11006

Marc Feldmann

Abstract not available at time of printing.

11003

Therapeutic potential of genetically modified T lymphocytes in Crohn's disease

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$CD4^+$ T-lymphocytes have a pivotal role in the pathogenesis of Crohn's disease. A specific IL-10-producing subset of $CD4^+$ T-lymphocytes (called Tr-1) potently down-regulates Th1-lymphocytes in experimental Crohn's disease. Hence, retroviral gene transfer of the anti-inflammatory cytokine interleukin-10 (IL-10) to peripheral blood T-lymphocytes is an attractive approach for the treatment of Crohn's disease. Human peripheral blood mononuclear cells were efficiently transduced using a vector containing IL-10-IRES-EGFP (IL-10-internal ribosomal entry site-enhanced green fluorescent protein) or a control vector containing EGFP (transduction efficiency between 30-40 %). Virtually all transduced cells were $CD3^+$, and either $CD4^+$ or $CD8^+$. $CD4^+$ and $EGFP^+$ double positive cells were sorted using fluorescence-activated cell sorting (FACS) assay achieving a 98% double positive population. Baseline production of IL-10 was between 400 and 700 pg/mL/10^6 $CD4^+$ T-lymphocytes transduced with IL-10-IRES-EGFP, whereas IL-10 was not detectable in the supernatants of EGFP transduced $CD4^+$ T-lymphocytes. $CD4^+$ T-lymphocytes stimulated with immobilized anti-CD3 and anti-CD28 monoclonal antibodies showed a fourfold increase of IL-10 production as compared to the control vector. Proliferation was reduced in the IL-10 transduced $CD4^+$ T-lymphocytes compared to the $EGFP^+$ control cells. Moreover, MHC class II was down regulated on the IL-10 transduced $CD4^+$ T-lymphocytes compared to the $EGFP^+$ control cells. The expression of the principal gut-homing integrin, $\alpha4\beta7$, was increased on the surface of transduced $CD4^+$ T-lymphocytes when compared to fresh $CD4^+$ cells. IL-10 transduced cells adhered more efficiently to MAdCAM transfected cells than to control cells. Human $CD4^+$ T-lymphocytes can be efficiently transduced to express functional IL-10, and these cells have gut homing properties. Experiments are in progress to examine the possibility of autologous grafting of these engineered cells in order to down-regulate mucosal inflammation in Crohn's disease.

11002

THERAPY WITH α-INTERFERON INDUCES IMPROVEMENT OF PLATELET COUNTS IN CHILDREN WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Objective: The aim was to investigate α-Interferon (IFN) in the treatment of children with chronic idiopathic thrombocytopenic purpura (ITP). **Method:** Inclusion criteria: patients with platelet counts (PC) < 50 x 10^9/liter and without treatment during the last month. Children received IFN (3 x 10^6 units/Kg/dose), SC, 3 times a week, during 4 weeks; if partial (< 150 x 10^9/liter) or no response was obtained, the same dose was administered for other 8 weeks. **Results:** Fourteen patients (aged 4 to 20 years), receiving 17 IFN courses, were included; all had received previous treatment with steroids and/or IgG, and one was splenectomized. Mean initial PC was 29±15 x 10^9/liter (10-50). A significant increase was achieved by 10 patients (71.4%), 7 of them reaching values > 150 x 10^9/liter. A PC > 50 x 10^9/liter was rapidly achieved (mean 12±7 days); the time required to reach the maximum PC was 38±38 days. All the responses were transitory: PC remained elevated throughout the treatment, but returned to initial values shortly after IFN discontinuation (mean 30±22 days). One patient had no change in PC, and another presented a drop of PC under basal values, but without bleeding. The "flu-like" syndrome was present in all the children during the first days. Neutrophil count decreased from 5.6±3.4 to 3.4±1.8x10^9/liter (*p: NS*) throughout the treatment. No other adverse effect was observed. **Conclusion:** IFN therapy induces a significant increase of PC in most of children and seems to be a valid alternative therapy.

11004

Predictive factors of sustained response to interferon alfa 2b and ribavirin therapy for chronic hepatitis C

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Our aim was to establish the favourable predictive factors of response to the combination of recombinant interferon alfa 2b (rIFNα-2b) and ribavirin (RV). **Methods:** We studied 50 patients with chronic hepatitis C included in a multicenter study. They received rIFNα-2b (3 MU, tiw, sc) and RV (1200 mg/d, orally). Eighteen of them (36%) obtained a sustained virological response. Age, sex, presence of cirrhosis, viremia levels, and HCV genotype were analyzed as basal predictive factors of such response. Negative HCV RNA at three months of treatment and reduction of RV dose were analyzed as predictive factors during the treatment. **Results:** The comparison of basal parameters between patients with and without sustained virological response showed: mean age 41±11 and 44±11 years (NS), age ≤ 35 years 44% and 19% [univariate analysis: P=0.05; multivariate analysis: P=0.13, odds ratio 2.9 (95%CI 0.7-11.3)], female 9 and 10 (NS), cirrhosis 11% and 9% (NS), mean viremia 5.8±7.2 and 7.5±12.7 Meq genome/ml (NS), viremia ≤ 2 Meq genome/ml 39% and 31% (NS), viremia ≤ 3.5 Meq genome/ml 50% and 59% (NS), and genotype non-1 50% and 16% [univariate analysis: P=0.009; multivariate analysis: P=0.03, odds ratio 4.7 (95%CI 1.2-18.6)], respectively. During the treatment, HCV RNA was negative at three months in 94% and 44% of patients (P=0.001) and RV dose was reduced in 67% and 56% (NS), respectively. **Conclusions:** Genotype non-1 and negative HCV RNA at three months of treatment were the predictive factors of sustained virological response. Age was only predictive in the univariate analysis.

26010

Interferon can Sensitize Cells to Viral-Induced Apoptosis by Modulating the Activity of the Death-Induced Signaling Complex (DISC).

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The interferons (IFNs) constitute a vital component of host antiviral defense by inducing a number of cellular genes, one of which is the dsRNA-dependent protein kinase, PKR. Interaction with dsRNA causes PKR to autophosphorylate and to catalyze the phosphorylation of substrate targets, the best characterized being the alpha subunit of eukaryotic initiation factor 2 (eIF2α) leading to the inhibition of protein synthesis in the cell. Substantial evidence also indicates that PKR may function as a second messenger for dsRNA signaling, to regulate the activity of NF-κB and even apoptosis. Using murine cell-lines that inducibly overexpress PKR, we demonstrate that activation of the kinase by dsRNA stimulated expression of the death receptor Fas and triggered apoptosis through FADD/caspase-8. Complementing this work using fibroblasts lacking key components of the apoptotic pathway, we found that influenza virus (WSN) also triggered apoptosis through the FADD death signaling pathway but that vesicular stomatitis virus (VSV) induced apoptosis predominantly through activation of mitochondrial-associated caspase-9. Significantly, treatment with type I IFN greatly sensitized a number of cell-lines to FADD-dependent apoptosis in response to dsRNA treatment or WSN infection but completely protected the cells against VSV replication in the absence of any cellular destruction. Our data infers that in addition to preventing viral replication by noncytopathic mechanisms, IFN can also sensitize cells to viral-induced apoptosis by regulating activity of the death-induced signaling complex [DISC] comprising FADD and caspase-8.

Functional polymorphism of cytokine genes

12002

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Abstract not available at time of printing.

12003

Linkage disequilibrium analysis of the IFN-γ chromosomal region in Sardinian simplex families with multiple sclerosis

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Type I and II interferons are generally thought to play important roles in multiple sclerosis (MS) pathology. Thus, the question arises whether genetic differences in the corresponding genes may affect the individual risk for developing MS.
We have previously demonstrated a significant association between an intronic CA dinucleotide polymorphism in the IFN-γ gene and MS in Sweden and Sardinia, using both case-control and family-based study designs.[1,2] In Sardinia, the IFN-γ polymorphism appeared to be associated with MS in those patients carrying '*low-risk*' HLA haplotypes (DR3-/DR4-). A CA polymorphism located in the IFN-α/β gene cluster was not associated with MS in Sardina and Sweden.[3] While these results are suggestive for a role of the IFN-γ gene in susceptibility to MS, additional analysis is needed in order to exclude the possibility that this effect is due to linkage disequilibrium with a neighbouring gene.
We have now analysed 5 other polymorphic markers surrounding *IFNG* on chromosome region 12q15 in 200 Sardinian simplex MS families. In addition, we have examined a recently reported potentially functional single nucleotide polymorphism in the *IFNG* 3' untranslated region. These data were processed by transmission disequilibrium and haplotype analysis and narrow down considerably the probable interval of localization of a susceptibility gene in the *IFNG* region.

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12004

THE DINUCLEOTIDE REPEAT POLYMORPHISM IN THE 3' FLANKING REGION OF THE IL-2 GENE IS ASSOCIATED WITH FREEDOM FROM ACUTE REJECTION

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To identify patients at risk for acute rejection (AR) after heart transplantation, we investigated two polymorphisms in the IL-2 gene. A single nucleotide polymorphism (SNP, T→G) located at position -330 in the promotor and a dinucleotide repeat $(CA)_n(CT)_m$ polymorphism (DNRP) in the 3' flanking region. Both polymorphisms were determined in DNA from patients (n=290) and controls (n=101). The genotype frequency of the SNP was comparable between patients and controls and no association was found between the SNP genotype distribution and AR (figure 1). For the DNRP, 15 alleles were observed in both our patient population and controls. In contrast to the SNP, an association between the DNRP genotype distibution and AR was found. The 135 allele was significantly more often present in patients without AR compared to patients with AR episodes and to controls (no AR: 34%; AR: 20% and controls: 19% p=0.02 and p=0.03, respectively, figure 2). However, there was no relation between the DNRP and the number of AR periodes (figure 2). We conclude that the 135 allele of the DNRP in the 3' flanking region of the IL-2 gene is associated with low immune responsiveness after clinical heart transplantation.

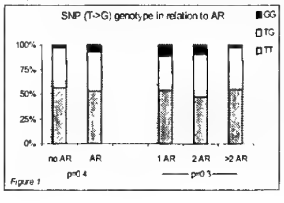

Figure 1

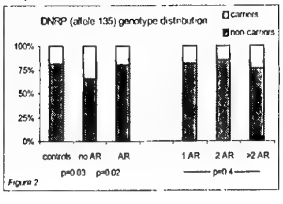

Figure 2

12005

Functional Polymorphisms in Growth Factors(EGF, PDGF-BB, VEGF)

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Growth factors, a family of multifunctional regulatory peptides, are synthesized by a number of different cell types. Many biological activities have been described for growth factors including mitogenesis, which is relevant to wound healing, tumourogenesis, cell proliferation, endothelial cell differentiation and also play important roles during embryonic development. We have identified a single nucleotide change (G→A) at position +61 from the transcription start site of the EGF gene and established PCR-RFLP analysis for typing purposes. Levels of EGF production *in vitro* were associated with the EGF genotype. Cells from A/A individuals produced significantly less EGF compared to cells from G/G ($p=0.003$) or heterozygous G/A ($p<0.001$) individuals. We also identified polymorphism (G→A) at position +286 from transcription start site of the PDGF-BB gene and developed ARMS-PCR for genotyping. *In vitro* production of PDGF-BB was examined. The production levels were correlated with the PDGF-BB genotype individuals, A/A individuals were significantly lower producers compared to cells from G/G and G/A ($p<0.0001$) individuals. We also looked at the correlation of the levels of VEGF produced *in vitro* and gene polymorphisms we have previously identified and established ARMS-PCR for genotyping analysis. Individuals C/C at position –2578 and G/G at position -1154 from transcription start site were significantly higher producers compared to cells from A/A at both loci ($p<0.01$) individuals. The polymorphism themselves may have regulatory function or alternatively, there is an allelic linkage between these polymorphisms and function polymorphisms elsewhere in the gene. All genetic variations described here may be of importance in a range of disease conditions.

12001

IL-4 PROMOTER GENE POLYMORPHISM IN HEART TRANSPLANTATION

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IL-4 is a cytokine of the Th2 subtype, secreted mainly by activated T-lymphocytes. IL-4 acts by down regulation of Th1 responses of macrophage and Th1 cells. On the other hand, IL-4 stimulates growth and differentiation of Th2 lymphocytes and mast cells. In transplantation Th2 cells are believed to be involved in induction of graft tolerance. Previous studies revealed that patients with a high frequency of IL-4 producing precursor T- helper cells (pHTL) before heart transplantation (HTX) had no or less rejection episodes compared to patients with low frequency of IL-4 producing pHTL (van Hoffen et al., Transpl Int. 13,S216,2000). Recently, single nucleotide polymorphisms (SNP) in the promoter regions of various cytokine genes have been identified. These SNP influence promoter strength and hence production of the cytokine. The promoter region of the IL-4 gene contains three SNP at positions –590 (C>T), -285 (C>T) and –81 (A>G) from the transcriptional start site. Three polymorphic alleles (resp T, T and G) increase promoter strength. We hypothesize that presence of these polymorphic alleles decreases rejection by increasing IL-4 production. Seventy HTX patients and 61 donors were genotyped for all three SNP by sequencing. SNP at –285 and –81 were not polymorphic in this study. We found 66 % CC, 30% CT and 4% TT at position –590 in patients and 80% CC, 18% CT, 2%TT in donors. Our results show that the incidence of rejection is significantly lower in patients receiving the T-allele from their donor compared to patients who did not ($\chi^2 = 4.52$, $p = 0.034$, relative risk = 3.31), regardless of the patients own haplotype. This indicates that IL-4 production within the donor heart and by cells from that donor is important. Potential candidates for this IL-4 production are various stromal elements, but also interstitial mast cells may be responsible.

12006

OLA: A RAPID METHOD FOR THE DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE PROMOTOR REGION OF CYTOKINES.

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Single nucleotide polymorphisms (SNP) in the promoter region of cytokines can influence the level of cytokine production. Individual differences in the level of cytokine production may influence the susceptibility to various diseases. SNPs can be detected by sequencing by which all nucleotides are identified. To detect known SNPs we developed Oligo Ligation Assay (OLA) for the promotor region of IL-10 and TGFβ in relation to heart graft rejection.
DNA was extracted from PBMC of 71 HTX patients and 61 healthy donors. PCR products were generated from the promoter regions. Each SNP requires three probes: a shared common probe, complementary to the sequence adjacent to the SNP and 3' end biotin labeled and 5' phosphorylated. Two allelic motif probes, complementary to either one of the 2 polymorphic nucleotides and 5' end labeled with either digoxygenin (DIG) or FITC. Only complementary strands can be ligated to the common probe, resulting in DNA strands 3' end labeled with DIG and 5' end labeled with either FITC or DIG. The products were detected by ELISA. Biotin was bound to the avidin coated wells and DIG detected by alkaline phosphate labeled anti-DIG, and FITC by peroxidase labeled anti-FITC. Analysis after developing the staining can be performed in a two-colour ELISA. The results were confirmed by sequencing of a limited number of cloned fragments and indicate that in donors a normal SNP distribution is detected. No strong correlation was found between the promoter SNP=s and rejection grades after heart transplantation.
We conclude that the OLA is a very specific and reliable technique to identify SNP=s and it is a cheap, fast and accurate method to screen large panels of patients.

12007

POLYMORPHISMS OF MARKERS WITHIN OR CLOSE TO GENES ENCODING CYTOKINES AND HEMOKINE RECEPTOR CCR5 AND ANALYSIS OF THE ASSOCIATION BETWEEN THEIR ALLELES AND MULTIPLE SCLEROSIS IN RUSSIA

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Several genetic factors are likely to play a role in the aetiology of multiple sclerosis (MS). We used a candidate gene strategy in a study of polymorphic markers within or close to genes encoding cytokines (tumor necrosis factor (TNF), lymphotoxin alpha (LT), transforming growth factor beta1 (TGF)) and CC hemokine receptor CCR5. One hundred fifty MS patients of Russian origin from Moscow region were investigated and compared to 150 healthy unrelated controls in the same population. All MS patients had definite MS according to Poser criteria. The following bi-allelic polymorphisms were investigated: G to A mutations in positions -308 and -238 of the TNF promoter region; the restriction fragment length polymorphism using restriction endonucleases NcoI and AspH1 in the first intron of LT gene; the mutation in the coding region of TGF gene resulting in Thr263→Ile polymorphism in the precursor protein; and the 32 bp deletion in the coding region of CCR5 gene. Length polymorphism of two highly polymorphous microsatellites TNFa and TNFb located in TNF/LT region was also analysed. There were no statistically plausible differences in the frequency of allelic forms of TNF, LT, TGF and CCR5 genes as well as of TNFb microsatellite. Stratification for the presence or absence of the MS-associated HLA class II DR2(15) haplotype did not influence the negative results. At the same time, the comparison of frequencies of distinct alleles of TNFa marker in MS patients and healthy controls showed the positive MS association with TNFa9 allele and the negative one with TNFa7 allele (corrected *p*-values <0.0001 and <0.002, respectively). The association of MS susceptibility with TNFa9 was independent of the presence of HLA-DR2(15) haplotype whereas the association with TNFa7 was revealed only in DR2(15)-bearing patients. We conclude that the studied alleles of the cytokine and CCR5 genes appear not to contribute to MS susceptibility whereas the MS association with TNFa microsatellite alleles is evidence of a role of some gene(s) within or close to TNF/LT locus in MS susceptibility.

12008

INTERLEUKIN-1 COMPLEX GENOTYPE REGULATES THE BLOOD IgA LEVELS IN HEALTHY MEN

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The immunoglobulin levels in blood are, at least to a great extent, genetically regulated, but the genes involved are unknown. The IgA class antibodies, in contrast to the other classes, are mainly produced by the mucosa-associated lymphoid tissue (MALT) and then secreted to the outer surfaces. The origin of IgA in circulation is not exactly known, but it may also be partly derived from MALT. There is strong evidence that the pro- and anti-inflammatory cytokines, such as IL-1, IL-6, IL-10 and IL-1Ra, regulate the strength of the inflammatory response on mucosal surfaces. The genes of these cytokines are polymorphic and the various alleles may differ in their capacity to produce the cytokine in question and therefore, these genes are possible candidates for regulators of IgA production. Now we analyzed the IgA, IgM and IgG blood levels in 176 healthy male blood donors and correlated these data to the known polymorphisms of the cytokines mentioned above. The data obtained demonstrate that the allele 2(T) of the IL-1β gene (C/T base exchange at the position –511) and allele 2 of the IL-1Ra gene (variable number of tandem repeats in intron 2) are associated with elevated levels of IgA in blood. No effect on IgG or IgM levels was observed. The increasing effect was more prominent in the levels of IgA2 subclass, i.e. the subclass mainly produced by the gut-associated lymphoid tissue. These results imply that the rate of "the base-line" inflammation on mucosal surfaces is genetically determined, resulting in variation of the blood IgA levels.

12009

FUNCTIONAL ASPECTS OF DISEASE-ASSOCIATED CYTOKINE GENE POLYMORPHISMS

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Variation in the host immune response plays an important role in disease Susceptibility and severity. Interindividual variation in cytokine production profiles has prompted speculation about the existence of a genetic basis for differential cytokine production levels. Studies with monozygotic twins have provided evidence for the existence of such a genetic basis in interindividual variation in the production of TNF and IL-10. Clinical relevance for this phenomenon was demonstrated by showing that familiar clustered cytokine production phenotypes were associated with outcome of meningococcal infection. For IL-13 deregulated production was observed in T cells from patients with allergic asthma. An extensive search for heterogeneity in the promoter regions of the TNF, IL-10 and IL-13 genes disclosed a number of polymorphisms. Some of these polymorphisms exhibited significant associations with disease and/or disease outcome. Experimental data, using promoter-reportergene studies, allele specific quantitation analysis and electrophoretic mobility shift assay, regarding the functional relevance of these disease associated polymorphisms using will be discussed.

Signal transduction II

13006

THE VARIED ROLES OF STAT3, FROM ANTI-INFLAMMATORY ACTION TO ONCOGENESIS

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STAT3 is activated in response to many stimuli, including IL6-type cytokines, IFNs, and growth factor receptor tyrosine kinases, and it is constitutively active in many human cancers, including the majority of head and neck cancers and mammary carcinomas. Gene targeting studies in mice showed that STAT3 is essential for an early stage in embryogenesis. We have used *loxP*-Cre recombinase conditional mutagenesis of STAT3 to evaluate its requirement in a number of pathways, including hematopoiesis, lymphoid development, adipogenesis, and malignancy. All normal hematopietic cell types can develop from bone marrow progenitors in the absence of STAT3, including a normal distribution of lymphoid, myeloid, and erythroid subsets. However, defects were detected in the ability to control inflammatory responses of myeloid cells, resulting in severe, fatal colitis due to massive infiltration of inflammatory cells in the intestinal epithelium accompanied by myeloid hyperplasia in the bone marrow and peripheral lymphoid tissues. These effects are due to the failure of essential, cytokine-mediated negative feedback required to control inflammatory responses to non-pathogenic stimuli. STAT3 is activated in growth factor-stimulated cells and is constitutively phosphorylated in v-src but not H-ras transformed fibroblasts. We find that STAT3-null fibroblasts are viable and responsive to growth factors; however, they are resistant to transformation by a number of oncogenes, including v-src and H-ras. Impaired transformation is due to a defect in proliferation under anchorage-independent growth conditions through an inability to maintain expression of cyclin D and A in the absence of integrin engagement. These results suggest two independent requirements for STAT3 in malignant growth, involving both growth factor and integrin-mediated response pathways, which may be only partially dependent on STAT3 tyrosine phosphorylation.

13012

NUCLEAR EXPORT OF THE STAT1 TRANSCRIPTION FACTOR

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Latent signal transducers and activators of transcription (STATs) reside in the cytoplasm but rapidly accumulate in the nucleus following cytokine stimulation. Nuclear accumulation requires specific tyrosine phosphorylation and STAT dimerization. The presence of STATs in the nucleus is transient, however, and within hours the STATs reappear in the cytoplasm. Results indicate that STAT1 can be dephosphorylated in the nucleus and actively exported by the CRM1 export receptor. CRM1 recognizes a specific amino acid sequence located within the DNA binding domain of STAT1. This region shares sequence and functional properties of characterized nuclear export signals, and a peptide corresponding to this region of STAT1 can function as a nuclear export signal. The location of this sequence within STAT1 suggests that it is not accessible to CRM1 when STAT1 is bound to DNA. If a STAT1 mutant that cannot bind DNA is evaluated, it is found to be able to translocate to the nucleus, but it is efficiently exported and does not accumulate in the nucleus. Evidence will be presented to support a model in which STAT1 is tyrosine dephosphorylated in the nucleus and subsequently dissociates from DNA, allowing recognition by CRM1 and nuclear export. The regulated export of STAT1 may contribute to silencing of the signal pathway and/or to re-establish STAT1 in the cytoplasm to monitor activity of receptor/kinase signals.

13008

Mechanism of cytoplasmic translocation of Stat1: Photobleaching analysis of Stat1-GFP

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Stimulation of Jak/Stat (Janus Kinase / Signal Transducers and Activators of Transcription) pathways with ligand results in Jak-dependent phosphorylation of the Stats at receptor complexes in the plasma membrane. Activated Stat dimers and possible associated proteins are translocated to the nuclear pore through which they are transported to function in the transcription of target genes.
FLIP (fluorescence loss in photobleaching) and FRAP (fluorescence recovery after photobleaching) were compared for GFP, PKC-GFP and cytoplasmic and nuclear Stat1-GFP in interferon-gamma-treated and control cells. Cytoplasmic movement of Stat1-GFP is extremely rapid and not inconsistent with a 'free diffusion' model for translocation of Stat1 from the receptor to the nuclear pore. Translocation and Stat1 function appear independent of continued Jak (receptor) activity and any requirement for an intact cytoskeleton.

13009

Transcriptional Synergy between IFNγ and IL-1 or TNF: Roles of STAT1 Serine Phosphorylation and IFNγ-Activated Phosphatidylinositol-3-Kinase.

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The synergistic action of IFNγ and TNF is central in maximizing the host inflammatory response to infection. At the molecular level, these cytokines synergize by amplifying the expression of genes involved in mounting the inflammatory response, primarily as a result of cooperation between IFNγ-activated STAT1 and TNF-activated NF-κB. The present study shows that maximum synergistic activation by IFNγ and TNF or IL-1 requires enhanced phosphorylation of STAT1 S727. Combination of these cytokines also results in synergistic activation of STAT1-driven transcription in T98G, HeLa or 2fTGH cells. Synergistic levels of IFNγ-inducible genes are decreased by about 60% in 2fTGH-based STAT1-null cells reconstituted with a mutant form of STAT1 containing a serine-to-alanine substitution, including the human guanylate binding protein-1 (hGBP-1) gene, whose synergistic activation by IFNγ and TNF does not involve NF-κB directly and is STAT1-dependent. Investigation of potential pathways contributing to STAT1-mediated synergy reveals that phosphatidylinositol-3-kinase (PI3K) plays an important role in STAT1 serine phosphorylation by IFNγ. Specific inhibition of PI3K in T98G or HeLa cells abrogates IFNγ-induced but not IL-1 or TNF-induced STAT1 S727 phosphorylation and reduces IFNγ-mediated gene activation by approximately 80%. IFNγ treatment of these cells stimulates PI3K activity which is associated with JAK1, and also activates the downstream PI3K effector Akt. STAT1 serine phosphorylation in response to IFNγ also requires JAK1, as it is lost in 2fTGH-based JAK1-null cells but is restored upon reconstitution with JAK1. Expression of constitutively active PI3K or Akt augments IFNγ-mediated STAT1-driven transactivation. Taken together, these results demonstrate vital roles of JAK1 and PI3K in IFNγ-mediated STAT1 activation, gene expression and transcriptional synergy.

13003

Quantification of STAT nuclear translocation in Intron® A stimulated Hela cells using an automated fluorescent imaging system.

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Numerous studies have demonstrated that interferons (IFN) activate the JAK-STAT signaling pathway. The Stats are recruited to activated cytokine receptors and once phosphorylated, translocate to the nucleus where they activate transcription of responsive genes. We describe a fluorescent cell based assay to quantify the nuclear translocation of Stat1 and Stat3 in response to interferon-α_{2b} (Intron® A) using the ArrayScan™ instrument and nuclear translocation imaging protocol developed by Cellomics™. Fluorescent images are acquired and the imaging protocol defines nuclear and cytoplasmic regions in individual cells. Measurement of Stat nuclear translocation is reported as the average cyto-nuclear difference, which is defined as the difference in fluorescence intensity between the nuclear and cytoplasmic regions. Intron® A stimulation of Hela cells elicited a dose and time dependent increase in both Stat1 and Stat3 nuclear translocation above basal levels. Stat1 nuclear translocation was observed at 15 minutes of treatment and reached maximum by 20-30 minutes. In contrast, Stat3 nuclear translocation was observed as early as 5 minutes and reached a maximum by 15 minutes. Both Stat1 and Stat3 exhibited a dose-dependent increase in nuclear translocation in response to Intron® A stimulation with a maximum response 2-fold above basal levels. IFN-γ stimulated Stat1 in a similar manner with a maximum response 3-fold above basal levels. In conclusion, we have demonstrated that IFN stimulated nuclear translocation of Stat1 and Stat3 can be rapidly quantified using the ArrayScan™ system.

13004

ERK5: A MAP KINASE ACTIVATED BY IFNα THAT PHOSPHORYLATES STAT1

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Activation of early response genes by interferons (IFNs) requires tyrosine phosphorylation of the Stat transcription factors. The protein sequences of Stats 1, 3 and 4 also contain a single, conserved MAP Kinase phosphorylation site (PX_nS/TP; P, proline; S/T, serine or threonine; X represents any amino acid and n=1 or 2) in the COOH terminus of the proteins. This site in Stat1 and Stat3 (serine 727) has been shown to be phosphorylated as a result of incubation of cells with a variety of cytokines including interferons. Phosphorylation of serine 727 is required for Stat1 to function as a maximal activator of IFNα and IFNγ stimulated genes. Several members of the MAP Kinase family have been implicated as regulators of serine phosphorylation of Stat1, but to date, none of these kinases have been shown to directly phosphorylate serine 727 of Stat1. Using a peptide that contains serine 727 of Stat1 as a substrate, we have isolated ERK5 as a kinase that can phosphorylate both the peptide as well as the intact protein. Furthermore, incubation of cells with IFNα stimulates ERK5 activity, and an IFNα stimulated Stat1-dependent reporter is inhibited by the expression of either dominant negative ERK5 or MEK5, (the upstream activator of ERK5). These results provide the first evidence for the role of ERK5 in IFNα signaling.

13010

Interferon induced gene activation utilizes histone and Stat2 acetylation and the components of TBP Free Taf Containing Complex (TFTC).

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Stat2, a part of the transcription factor complex ISGF3, is involved exclusively in gene activation following type I IFNs binding their receptors. We have shown that Stat2 is a potent transcriptional activator. In order to elucidate the mechanism for this potent activation, we investigated potential target components of the basal transcription machinery. We found that $Taf_{II}130$, but not $Taf_{II}28$ or $Taf_{II}100$, can act as a coactivator for ISGF3 transcriptional activation. The $Taf_{II}130$ requirement in the interferon response was shown to be independent of the histone acetyl transferase (HAT) catalytic activity of $Taf_{II}250$. However, other HAT containing proteins can interact with the transactivation domain of Stat2, including p300/CBP, P/CAF, and hGCN5. Surprisingly, an alanine mutation in Stat2 that inhibits the recruitment of p300 is as transcriptionally active as the wild type protein. However, a mutation in Stat2 that blocks GCN5 binding renders Stat2 transcriptionally inactive and has a dominant negative effect on interferon induced gene activation. Moreover, a truncated version of GCN5 acts as a dominant negative inhibitor of ISGF3 transcriptional activation. Importantly, HAT activity copurifies with Stat2 as shown in an in vitro HAT assay. The pattern of free histone acetylation resembles the specificity for GCN5 family members. Finally, Stat2 is acetylated on a lysine residue in an interferon dependent manner. The selective recruitment of $Taf_{II}130$ and the involvement of GCN5 enzymatic activity suggest that TBP-free Taf containing complex (TFTC) may be specifically utilized in the IFN response and that both histone as well as factor acetylation are involved in the regulation of interferon inducible genes.

Type I interferons: selective signalling and effects on the nervous system

14004

Cytokines and neurotrophins in neuroinflammation; impact of non-MHC genetic regulation

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We study two forms of neuroinflammation in the rat. 1) myelin antigen induced autoimmune neuroinflammation in the central and peripheral nervous system. Hereby, myelin oligodendrocyte glycoprotein (MOG) induced experimental autoimmune encephalomyelitis (EAE) mimics MS closely and may therefore disclose mechanisms of importance for human disease. 2) Innate immunity, represented by the glial cell activation around motoneurons which to a large extent die after ventral nerve root avulsion (VRA). This experimental paradigm models neurodegenerative disease and its accompanying inflammation.
To find evolutionary selected genetically regulated pathways of importance the outcome of neuroinflammation, we have mapped genome regions in F2 crosses between EAE susceptible and resistant strains. Sofar we have found 9 different non-MHC genome regions in the rat regulating EAE outcome. Several of these co-localize with genome regions regulating inflammation in other organs,such as joints. Some of them regulate T1/T2 bias as detected by antibody isotypes or cytokine expression. In all cases a T1 bias promote disease. Neuron death, cytokine production and degree of glial activation after VRA is also subject to genetic regulation by non-MHC genes, which now can be sought in mapping studies. Interestingly, EAE induced concommittantly with VRA results in motoneuron rescue. The protective aspect of the neuroinflammation resides in bystander recruited lymphoid cells producing neurotrophins. These are shown to be constitutively expressed in systemic lymphoid cells. Their role as cytokines in the immune system will now be interesting to explore.

14001

IFN beta-1a in Relapsing-Remitting Multiple Sclerosis patients: analysis of IFN-induced proteins and antibodies to IFN during 12 months of therapy.

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In vivo studies on healthy volunteers have shown that the measurement of IFN-induced proteins may be useful to study the pharmacodynamics of IFN beta. However, such issue have been addressed only marginally in patients and, above all, in Relapsing Remitting Multiple Sclerosis (RRMS) patients. Then, aim of the study was to define in a longitudinal study the pharmacodynamics of IFN beta-1a in RRMS treated patients. The frequency of binding and neutralizing therapy-induced antibodies to IFN beta 1a have been also evaluated. Thirty RRMS patients with a mean disease duration of 4.3 years and mean EDSS of 1.3 are treated with IFN beta-1a (Avonex, Biogen), 6 MIU i.m. once a week for 12 months. Patient samples were collected at baseline, 24 and 48 hrs after the injection of IFN beta-1a at the time 0 (beginning of the study) and then at 3, 6, 9 and 12 months of therapy. Levels of neopterin, beta2 microglobulin (B2m), MxA- mRNA, IFN gamma, circulating IFN beta, neutralizing (N) and binding antibodies (abs) to IFN beta are being analyzed. So far, results on circulating levels of neopterin, B2m, IFN beta and Nabs to IFN beta are available.The results on neopterin examination indicate that there is: a significant decrease in the baseline expression at 9 and 12 months of therapy ($p<0.005$); a significant difference between the increase at time 0 (2.4±0.9) compared to those observed at time 3 and 6 months of therapy (1.6±0.6 and 1.7±0.6, respectively) ($p<0.05$). As far as B2m is concerned the data suggest that a significant increase can be recorded after 24/48 hrs from each IFN administration and that there is decrease of the IFN-induced levels of the protein at 12 months of therapy. Only modest differences in B2m baseline values were found during the therapy. Nabs development has been detected in 2 patients (6,6%) after 6 months of therapy, while circulating IFN beta was undetectable by a bioassay (< 8 IU/ml) at each time point tested. The biological meaning of these findings as well as their relevance from the clinical and therapeutical point of view are still to be addressed. New insights will be gained when the measurement of the other IFN-induced proteins as well as the final clinical examination of the patients will be performed.

14006

ENFORCED EXPRESSION OF JAK1 IN NEURONS RESCUES THE LETHALITY OF THE JAK1 DEFICIENT MOUSE.

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$Jak1^{-/-}$ mice, generated previously in our lab, are runted at birth, fail to nurse, and die perinatally. Three observations suggested that a neuronal defect was responsible for this early lethality. (1) Sensory neurons from $Jak1^{-/-}$ mice fail to survive when cultured in vitro with neurotrophic cytokines such as IL-6, LIF and CNTF, (2) mice deficient in CNTFRα or LIFRβ exhibit a gross perinatal lethal phenotype that is similar to $Jak1^{-/-}$ mice, and (3) CNTFRα, which requires Jak1 for signal transduction, is restricted in its expression to neuronal tissue and skeletal muscle. To test this hypothesis, Jak1 was transgenically expressed in $Jak1^{-/-}$ mice using a construct driven by the neuron specific enolase promoter to generate $Jak1^{-/-}NE^{+}$ mice. Unlike $Jak1^{-/-}$ mice, $Jak1^{-/-}NE^{+}$ mice suckle and thrive in our facility. IP/Western blot and RT-PCR analyses of tissue samples from the rescued mice confirmed that Jak1 expression was limited to neuronal tissue and testis. Adult $Jak1^{-/-}NE^{+}$ mice retain all the immunologic deficits observed in the neonatal $Jak1^{-/-}$ mice. They display a major defect in T and B lymphocyte development that is more severe than that reported for mice that lack either Jak3, γc receptor subunit, or IL-7. $Jak1^{-/-}NE^{+}$ mice as old as 8 months do not develop splenomegaly as do mice deficient in other components of the IL-2 signaling pathway. Moreover, cells from $Jak1^{-/-}NE^{+}$ mice are unresponsive to IFNα/β and IFNγ. Thus, Jak1 fulfills critical roles in the development and/or function of both the nervous system as well as the immune system. The rescued $Jak1^{-/-}NE^{+}$ mouse should prove to be a novel in vivo model where a single genetic mutation leads to a combined deficiency of both innate and adaptive immunity.

14002

DIVERGENT SIGNALING PATHWAYS MEDIATE BENEFICIAL/ PROTECTIVE VERSUS TOXIC ACTIONS OF IFN-alpha TRANSGENICALLY EXPRESSED IN THE MOUSE CNS

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Transgenic (termed GIFN) mice with astrocyte-targeted expression of IFN-alpha are protected from infection with neurotropic viruses but develop a transgene dose-related progressive neurodegenerative disease with inflammation and pronounced calcification in association with upregulated expression of a number of IFN-regulated genes such as PKR and OAS. Here we examined the regulation and the role of the key IFN-signaling molecule STAT1 in mediating the biologic actions of IFN in the CNS. CNS levels of STAT1 RNA and protein were low in the brain of wild type mice but increased significantly in a transgene dose-related fashion in GIFN mice with high expression localized to neurons in various brain regions. Many positive neurons exhibited nuclear localization of STAT1 protein indicative of transcriptional activation. Consistent with this, expression of the OAS gene in the CNS and the GIFN mice showed broad overlap with the expression of STAT1. Surprisingly,GIFN mice deficient for STAT1 had accelerated and more severe neuropathological alterations than STAT1 replete GIFN mice. CNS expression of the IFN-regulated genes including PKR, OAS and TGTP as well as the other members of the IFN-signaling pathway, STAT2 and IRF9, was not increased in GIFN/STAT1 KO mice consistent with the absence of STAT1-dependent IFN-regulated gene transcription. We conclude 1) STAT1 gene expression is highly regulated in the CNS, particularly in neurons, 2) STAT1-dependent IFN-regulated gene transcription plays a protective role against the neurotoxic actions of IFN-alpha and 3) the neurotoxic actions of IFN-alpha are mediated by an as yet unidentified alternative signaling pathway. Supported by NIH MH47680 and MH62231.

14003

EXTRACELLULAR RELEASE OF MULTIPLE SCLEROSIS-ASSOCIATED RETROVIRUS (MSRV) *IN VIVO* AND *IN VITRO* AND CYTOKINE PRODUCTION BY SARDINIAN MS PATIENTS AND HEALTHY HUMANS

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Multiple sclerosis-associated retrovirus (MSRV) is an exogenous member of the HERV-W human endogenous retrovirus family, produced by multiple sclerosis (MS) patients, both *in vivo* and *in vitro*. The italian Sardinia island has one of the highest MS frequencies world-wide, and an MS assoiation with HLA-DR3 and -DR4 haplotypes. To search for factors accounting for this anomalous high incidence, the production of extracellular MSRV *in vivo* and *in vitro* by Sardinian MS patients and matched healthy donors was studied, as well as the *in vitro* production of cytokines potentially related to MS pathogenesis. Blood samples were processed for extracellular RNA extraction, reverse transcription and nested polymerase chain reaction amplifications with primers specific for MRSV-*pol* gene. Untreated and activated peripheral blood mononuclear cells (PBMC) were cultured for cytokine production and virus release. Results indicate a striking correlation between presence of extracellular MSRV in blood and MS disease, but the virus is produced also by healthy donors (100% and 12%, respectively, $p<0.000001$). MSRV is increasingly produced with time by cultured PBMC, and its release is regulated by cell activation and cytokine treatments. PHA-activated PBMC from MS patients with the DR4(+)/DR3(-) haplotype produce significantly higher amounts of TNFα and IFNγ than cells from DR3(+)/DR4(-) patients. In addition, homozygous DR3(+) PBMC from MS patients produce significantly lower amounts of IL-10 than those from homozygous DR3(+) healthy donors. It is unclear to date whether MSRV exerts any pathogenic role in MS, or it is simply an epiphenomenon; at least it might constitute a diagnostic marker. The abnormal production of detrimental or regulatory cytokines might account for the genetic susceptibility to MS of different HLA-subgroups of Sardinian MS patients.

09005

STRUCTURE-FUNCTION ANALYSIS OF THE BINDING OF TypeI INTERFERONS AND THEIR RECEPTORS.

Gideon Schreiber, Laila Roisman and Jacob Piehler

Type I interferons (IFN's) exert pleiotropic activities by binding to two cell surface receptors, ifnar1 and ifnar2. We are investigating the biophysical basis of IFN signaling by characterizing the complex of the extra-cellular domain of ifnar2 (ifnar2-EC) with IFNs on the level of purified recombinant proteins *in vitro*. Here, we present a detailed mutational study which revealed the functional epitopes on both IFN and ifnar2. Kinetic and thermodynamic parameters of the various mutants were determined by label-free heterogeneous phase detection. Previous studies on IFNα2 have identified the AB-loop and D-Helix as being involved in ifnar2 binding. Here we show that residues located on the A and E-helixes significantly contribute towards ifnar2 binding, with most of the hot-spot residues being located on the E-helix. According to this model of interaction the functional epitope towards ifnar2 consist of a flat surface, with the E-Helix being at its center, flanked by the AB-loop and the A-Helix. The contribution of the D-Helix towards ifnar2 binding was found to be marginal. Relating the affinity of the various IFN mutations towards ifnar2 with their ability to induce an anti-viral state or to inhibit cell proliferation, revealed a simple linear relation between binding affinity and biological activity. This suggests that the formation of the IFN-ifnar2 complex is the rate-limiting step for IFN signaling. On ifnar2, only residues located on three loops of the first immunoglobulin like domain (45-55, 77-82 and 102-106) were identified as hot-spots for the interaction with IFNα2. Based on this data, the functional epitope was mapped on a modeled structure of ifnar2, which was constructed from its homology with other members of the class II hCR family. To further characterize the IFNα2 - ifnar2 interface, a large number of double-mutant cycles were constructed between residues on both proteins. This analysis revealed the identity of a number of residues which interact between the two proteins, suggesting a specific structural mode of interaction between the two. IFNα2 and IFNβ bind competitively to the same functional epitope on ifnar2. However, mutational analysis revealed distinct centers of binding for these IFNs on ifnar2, which may be related to differences in cellular activation between them.

26008

DISTINCTIVE *IN VITRO* AND *IN VIVO* EFFECTS OF IFN-α AND IFN-β IN EWING'S SARCOMA

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Ewing's sarcoma, a childhood bone tumor of neuroectodermal origin is characterized by a t(11 :22) chromosomal translocation resulting in the fusion of the 5' end of EWS gene (Ewing's sarcoma gene) with the 3' portion of the chromosome 11-derived Fli-1 gene carring the Ets domain with a specific DNA-binding motif. Four human cell lines derived from Ewing's sarcoma (EW-7, EW-1, COH and ORS), were investigated to establish the effects of human recombinant IFN-α2 and IFN-β on cell proliferation and apoptosis. All four cell lines were much more sensitive to the antiproliferative effects of IFN-β than of IFN-α.. Analysis of the early signals triggered by IFN-α and IFN-β demonstrated the two IFNs were similarly effective in inducing tyrosine phosphorylation of the Jak-1 and Tyk-2 kinases and the transcription factors Stat-1 and Stat-2. Interestigly, an additional rapid phosphorylation of Stat-1 on serine 727 was observed after IFN-β treatment, with concomitant activation of p38 MAP kinase. IFN-β induced the formation of the ISGF3 complex more efficiently than IFN-α, as well as sustained induction of IRF-1, which may account for its greater induction of 2'5' oligo(A)synthetase and greater inhibition of cell proliferation. IFN-β, but not IFN-α, induced apoptosis in wild-type p53 EW-7 and COH cell lines appeared to be mediated by IRF-1 and involved the activation of caspase-7. Ectopic expression of IRF-1 induced apoptosis in all four cell lines which correlated with the activation of caspase-7 and with the downregulation of the Bcl-2 oncoprotein, as observed for IFN-β induced apoptosis in parental EW-7 and COH cell lines.
EW-7 cells were established as transplantable xenografts into nude mice. Tumors, (12 mice per group) were treated as soon as they reached a 60-100 mm3 volume by intratumor injection of 8×10^5 units of human recombinant IFN-α or IFN-β (5 days per week). The statistically significant tumor growth inhibition was 43 % and 65 % for IFN-α and IFN-β treatment respectively. These results provide a rational basis for a promising therapeutic approach of Ewing's sarcoma.

10040

A ROLE FOR NF-κB IN THE INDUCTION OF CHEMOKINE CXCL11 BY IFN-β

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The β-R1/I-TAC (CXCL11) gene encodes an α-chemokine that is a potent chemoattractant for activated T-cells. Previous experiments in cell lines that lacked individual type I IFN signaling components suggested that induction of β-R1 by IFN-β required transcription factor ISGF-3 and an additional component. We now provide evidence that NF-κB can serve as this component. *In vivo* genomic footprinting of the β-R1 5'flanking region revealed transient IFN-β-induced dimethyl sulphate hypersensitivity at residue -98 of a canonical NF-κB binding site. Examination of two sibling non-transformed fibroblast cell lines indicated that induction of β-R1 by IFN-β was only observed in ataxia telangiectasia (ATM) cells that expressed detectable basal nuclear NF-κB activity, composed of p65/p50 heterodimers. Site-directed mutagenesis of an NF-κB binding-site in the β-R1 promoter abrogated induction of a β-R1 promoter-reporter by IFN-β but not IFN-γ, as assessed by transient transfection assays in HT1080-derived fibrosarcoma cells. Over-expresssion of IκBα blocked induction of a β-R1 promoter-reporter by IFN-β, but did not affect induction of a Type I IFN induced 561 promoter-reporter. These results suggest that NF-κB is involved in the signaling pathway that supplements ISGF-3 for induction of β-R1 by IFN-β.

26009

CHARACTERIZATION OF A NOVEL DEATH REGULARTORY GENE INVOLVED IN INTERFERON-β AND RETINOIC ACID INDUCED CELL DEATH

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Interferons (IFNs) are a group of multifunctional cytokines that stimulate antiviral, antitumor, and immunoregulatory activities by activating gene expression though the JAK-STAT signal transduction pathway. Retinoic Acid (RA) is a Vitamin A derivative that has strong influence on cell growth and differentiation. Although either IFN or RA alone can inhibit transformed cell growth, they cause a synergistic growth suppression when used in combination. We show that treating breast tumor cells with the combination of IFN-β and RA can induce cell death *in-vitro* and *in-vivo*. Using a genetic method called Suppression of Mortality by Antisense Rescue Technique (SMART), we have identified several Genes associated with Retinoid and Interferon induced Mortality (GRIM). In this study we have characterized the novel death regulatory gene GRIM-19. GRIM-19 encodes a 16 kDa protein that is expressed in a tissue specific manner and is localized predominantly in the nucleus. The expression of GRIM-19 is induced by the combination of IFN and RA treatment in several breast tumor cell lines. Overexpression of GRIM-19 in breast tumor cells suppresses cell growth. Cells that overexpress moderate levels of GRIM-19 are highly sensitive to IFN/RA combination treatment. GRIM-19 has been mapped to human chromosome 19. This region of chromosome 19 is essential for prostate tumor suppression.GRIM-19 homologues are present in other organisms. These data together establish GRIM-19 as a novel tumor suppressor gene functioning under the control of Interferons and Retinoids.

Cytokines and interferons in hemopoiesis and angiogenesis

15003

REGULATION OF ANGIOGENESIS BY INTERFERON TYPE I

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The progressive growth of neoplasms and the production of metastasis are dependent on angiogenesis. The extent of angiogenesis depends on the balance between proangiogenic and antiangiogenic molecules. The growth of many human neoplasms is associated with the absence of the endogenous inhibitor of angiogenesis, interferon (IFN). We found that IFN-α or -β (but not IFN-γ) downregulate transcription and protein production of bFGF and collagenase type IV in human carcinoma cells by mechanisms independent of cell proliferation. Detailed immuno-histochemical analysis revealed that IFN-β is produced by nondividing, differentiated cells but not by normal dividing cells nor by carcinoma cells. IFN-α has been widely used alone or in combination with other agents to inhibit cell proliferation and, hence, investigators have utilized high doses given 2-3 times per week. Recent data from our laboratory show that optimizing dose and schedule of IFN-α is necessary for maximal downregulation of angiogenesis-related genes and, hence, inhibition of angiogenesis in neoplasms. Maximal biological effects of IFN-α required daily administrations of low doses. In fact, administration of maximal tolerated dose of IFN-α failed to inhibit angiogenesis due to a feedback mechanism leading to induction of suppressors of cytokine signalling (SSI). Thus, efforts to treat patients with maximal tolerated doses of IFN-α may be counterproductive. Clinical trials using optimal biological dose of IFN-α to inhibit tumor cell invasion and angiogenesis are underway.

15001

CELL SURFACE-EXPRESSED MOESIN REGULATES T CELL INTERACTIONS WITH TISSUE COMPONENTS AND BINDS ADHESION-MODULATING IL-2 PEPTIDES GENERATED BY ELASTASE

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The adhesion and migration of leukocytes through the extracellular matrix (ECM) to inflamed tissues depend on adequate responses of the cells to variations in the chemical composition of their milieu and the quantity of signals, as well as on the functioning of various cytoskeletal and cell surface elements. Ezrin, radixin, and moesin form a family of proteins that link the plasma membrane to the actin cytoskeleton. However, the possible surface expression of the extension spike protein moesin on T cells, as well as its role in cell adhesion, has not been fully elucidated. Recently, we demonstrated that pM amounts of IL-2 peptides generated by elastase, abrogate the adhesion of activated T cells to ECM proteins. Here, we further examined the adhesion-regulatory effects of these peptides and the existence of an IL-2 peptide-specific receptor on T cells. We found that when presented to T cells in the absence of another activator, the IL-2 peptides induced cell adhesion to vessel wall and ECM components. Binding of radiolabeled IL-2 peptides to T cells, precipitation with immobilized peptides, and amino acid sequencing of the precipitated proteins revealed that these peptides exert their functions via a cell surface-expressed 78-kDa moesin. This notion was further supported by our findings that (i) anti-moesin mAb inhibited the binding of human T cells to immobilized IL-2 peptides, (ii) recombinant moesin bound the three IL-2 peptides, and (iii) soluble recombinant moesin inhibited the IL-2 peptide-induced T cell adhesion to fibronectin. Interestingly, moesin appears to be involved in modifying T cell behavior in tissues in response to adhesion-regulating signals (other than the IL-2 peptides), since T cell adhesion to fibronectin and hyaluronic acid induced by various mediators was inhibited by mAb anti-moesin.

15004

SITE-SPECIFIC SERINE PHOSPHORYLATION OF THE IL-3 RECEPTOR IS REQUIRED FOR HEMOPOIETIC CELL SURVIVAL.

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The balance between cell survival and death is controlled by transmembrane receptors which upon ligand stimulation undergo phosphorylation and cause downstream activation of anti-apoptotic molecules. In the hemopoietic compartment, a prototypic system undergoing constant cell renewal, IL-3, GM-CSF and IL-5 receptors are major transducers of survival signals, however, the receptor proximal events that determine this vital function have not been defined. We have found that IL-3 stimulation induces phosphorylation of Ser-585 in the common β chain (β_c) of these receptors, and that this event is mediated by cAMP-dependent protein kinase (PKA). This promotes the association of phospho-Ser585 of β_c with the phosphoserine-binding adaptor protein 14-3-3 and the p85 subunit of PI 3-kinase leading to activation of this kinase and its down-stream signalling partner, Akt. Mutation of Ser585 severely impairs this signalling pathway without affecting activation of STAT5, MAPK, JNK or the recruitment of SHP2. Significantly, mutation of Ser585 greatly reduces cell survival in response to IL-3 and promotes apoptosis without affecting entry into the cell cycle or c-myc induction. These results define a distinct IL-3 receptor-mediated survival pathway regulated by site-specific receptor serine phosphorylation and 14-3-3 binding, and suggest that this novel mode of signalling may be utilized by disparate transmembrane receptors that have as a common theme the transduction of survival signals.

15002

IP-10 INDUCTION AND INHIBTION OF ANGIOGENESIS BY THE ANTITUMOR AGENT 5,6-DIMETHYLXANTHENONE-4-ACETIC ACID (DMXAA)

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5,6-Dimethylxanthenone-4-acetic acid (DMXAA), a drug synthesized in this laboratory which halts tumor blood flow and induces tumor hemorrhagic necrosis in transplantable murine tumors, is known to induce the synthesis of antiangiogenic cytokines *in vitro*. We have measured the induction of mRNA for modulators of angiogenesis *in vivo*, and investigated whether DMXAA may also have an additional antiangiogenic action through the production of these cytokines. The genes for interferon-α and for IP-10 were strongly induced in both spleen and Colon 38 tumor tissue following DMXAA treatment, while that for interferon-γ (IFN-γ) was induced in spleen but not in tumor. Expression of mRNA for interferon-β, and for the p35 or the p40 subunits of IL-12, was not observed in either tissue. IP-10 gene induction was not a result of IFN-γ production induced with DMXAA since spleen and Colon 38 tumor tissue from DMXAA-treated mice that lacked functional IFN-γ receptors (IFN-$\gamma R^{0/0}$) expressed similar amounts of IP-10 mRNA as those from wild-type mice. A single intraperitoneal injection of DMXAA (20 mg/kg) was sufficient to reduce fibroblast growth factor (bFGF)-induced endothelial cell invasion of Matrigel implants in athymic nude mice by nearly 100 percent. Antibodies to IP-10 reduced DMXAA's inhibition of endothelial cell invasion by 60%, whereas antibodies to TNF-α and IFN-γ neutralised only 5% of DMXAA's action and antibodies to IFN-α had no effect. The present data support the hypothesis that DMXAA may exert an *in vivo* antiangiogenic effect, mediated largely by its induction of IP-10.

15006

WOUND HEALING IN MIP-1$\alpha^{-/-}$ AND MCP-1$^{-/-}$ MICE

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A salient feature of normal wound healing is the development and resolution of an acute inflammatory response. While much is known about the effector functions of inflammatory cells within wounds, little is known about the chemotactic and activation signals that influence this response. As previous investigations have demonstrated that the CC chemokines MIP-1α and MCP-1 are abundantly produced in acute wounds, wound healing was examined in MCP-1$^{-/-}$ and MIP-1$\alpha^{-/-}$ mice. Surprisingly, the degree of re-epithelialization, angiogenesis and collagen synthesis in the wounds of MIP-1$\alpha^{-/-}$ mice was nearly identical to controls. In contrast, MCP-1$^{-/-}$ mice displayed significantly delayed repair. The kinetics of re-epithelialization were dramatically altered, with the greatest delay at day 3 after injury (28 ± 5% re-epithelialised vs. 79 ± 14% re-epithelialised, MCP-1$^{-/-}$ vs. wt, $p<0.005$). Wound angiogenesis was also delayed in MCP-1$^{-/-}$ mice, with a 48% reduction in capillary density at day 5 post-injury. Collagen synthesis was impeded as well, with the wounds of MCP-1$^{-/-}$ mice containing significantly less hydroxyproline than wt (25 ± 3 μg /wound vs. 50 ± 8 μg /wound in wt at day 5, $p<0.0001$). Despite the delayed in healing, there was no change in macrophage numbers in wounds from MCP-1$^{-/-}$ mice versus wt. Thus, MCP-1 appears not to serve as a major monocyte chemoattractant at sites of acute injury. Rather, MCP-1 may influence the activation or effector state of macrophages and other cell types within the healing wound. Taken together, the results demonstrate that MCP-1 plays a critical role in wound healing.

15005

MxA AND β-DEFENSIN-2, TWO ANTI-INFECTIVE PROTEINS INDUCIBLE BY IFNα/β AND CYTOKINES, RESPECTIVELY, ARE CONSTITUTIVELY EXPRESSED IN MUCOSA AND UP-REGULATED IN LESIONAL AND HEALING SKIN.

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MxA is a cytoplasmic 76 kD GTPase that accumulates to high levels in cells in response to IFNs α and β, to certain viral infections, and to dsRNA. The MxA protein inhibits the replication of certain RNA viruses. Human β-defensin-2 (HBD-2) belongs to a family of small cationic, cysteine rich peptides, which display antimicrobial activities against a broad spectrum of bacteria, yeast and fungi. HBD-2 has been isolated from psoriatic scales and is inducible in skin by microorganisms and by inflammatory cytokines, such as TNF-α and IL-1β. By using standard immunohistochemical techniques we have found that the regenerating epidermis of healing wounds produces both MxA and HBD-2, whereas the epidermis of non-wounded human skin does not. We have also observed that both proteins are expressed at mucosal surfaces (human foreskin). In addition, MxA and HBD-2 are detectable in the epidermis of Apligraf® - a bioengineered bi-layered living human skin construct, which is produced from *in vitro* propagated neonatal human foreskin cells. Since Apligraf® is manufactured under strict aseptic conditions and the cell banks and biological compounds used for its production are subject to rigorous microbiological safety tests, it is unlikely that the induction of MxA depends on signals produced by viral infection. It is interesting to notice that both MxA and HBD-2 genes have NF-κB sites in their promoter region, since NF-κB has been shown to be activated in wounded keratinocyte cultures, and to be involved in the upregulation of several proteins known to be expressed in epidermal lesions (e.g. MMP-9, keratin 6, and IL-8). The presence of an inducible intrinsic anti-infective mechanism in the epidermis might explain why superfical epidermal wounds usually heal without major complications despite the loss of epidermal barrier and subsequent exposure to opportunistic infections.

Interferon-inducible proteins (includes PKR)

16003

A ROLE FOR PROTEIN KINASE PKR IN p38 MAPK ACTIVATION AND THE INNATE IMMUNE RESPONSE TO BACTERIAL ENDOTOXIN.

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Protein kinase RNA-regulated (PKR) is a serine threonine kinase that is induced by type I interferons (IFNs), activated by double-stranded (ds) RNA and recognized for its role in the innate antiviral response. More recently, PKR has been demonstrated to play different roles in the cell, including signal transduction in response to cellular stress activators. The relationship between PKR and the stress-activated kinases (SAPKs), p38 and c-Jun N-terminal kinase (JNK) is not clear. Using mouse embryo fibroblasts (MEFs) from isogenic PKR-wild-type and null mice, we established a requirement for PKR in the activation of SAPKs by dsRNA, lipopolysaccharide (LPS), tumor necrosis factor (TNF)-alpha, interleukin (IL)-beta and IFN-gamma. This does not reflect a global failure to activate SAPKs in the PKR-null background as these kinases are activated normally by anisomycin and other physiochemical stressors. Activation of p38 was restored in immortalized PKR-null fibroblasts by reconstitution with human PKR. We also show that levels of IL-6 and IL-12 are diminished in LPS-treated PKR-null MEFs, and that production of these cytokines is also impaired in PKR-null mice challenged with LPS. Our findings indicate, for the first time PKR is required for p38 signaling and plays a potentially important role in the innate immune response to bacterial endotoxin.

16001

ESSENTIAL ROLE OF THE dsRNA-DEPENDENT PROTEIN KINASE, PKR, IN INNATE IMMUNITY TO VIRAL INFECTION.

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The interferons (IFNs) comprise an essential element of innate immunity to viral infection by inducing a large amount of cellular genes that serve to directly inhibit viral replication and to initiate and direct subsequent adaptive immune responses. Although many of the key IFN-inducible genes remain to be identified and/or characterized, one such gene product referred to as the dsRNA-dependent protein kinase, PKR has been proposed to play a critical role in IFN-mediated antiviral function. Interaction with dsRNA causes PKR to autophosphorylate and to catalyze the phosphorylation of substrate targets, the best characterized being the alpha subunit of eukaryotic initiation factor 2 (eIF2α), leading to the inhibition of protein synthesis in the cell. However, substantial evidence also indicates that PKR may function as a second messenger for dsRNA signaling, to regulate the activity of NF-κB and even apoptosis. Here, we demonstrate the importance of PKR in antiviral host defense by showing that mice lacking PKR are extremely susceptible to intranasal (i.n.) infection with vesicular stomatitis virus, VSV. Interestingly, IFN treatment protected fibroblasts lacking PKR to VSV infection, indicating the induction of alternate antiviral genes, but failed to protect intranasally infected PKR knockout mice, suggesting a suppressed IFN system in the respiratory tract. Aside from being sensitive to VSV infection, PKR-deficient animals were found to be ten times more sensitive to lethal respiratory infection with influenza virus (WSN). High level viral replication and significant viral-induced apoptosis in tissues was detected in the pulmonary tract, as well as in other organs. Collectively, our data indicate that the IFN-inducible PKR is an essential and non-redundant component of innate immunity that acts early in host defense prior to inception of the acquired immune response.

16006

A ROLE FOR THE INTERFERON (IFN)-INDUCIBLE DOUBLE-STRANDED RNA-ACTIVATED PROTEIN KINASE PKR IN THE INDUCTION OF IFN AND OTHER PROINFLAMMATORY CYTOKINES.

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Protein kinase PKR is a ubiquitous serine/threonine kinase that was initially identified as a translation inhibitor, due to its effects on initiation factor eIF2-α, in antiviral pathways mediated by IFNs. Previous reports including ours have demonstrated that PKR is inducible by cellular stress signals including TNF-α, ds-RNA, and endotoxin. We have also shown that suppression of PKR expression results in the establishment of persistent virus infection associated with reduced virus-induced apoptosis and expression of IFN (PNAS 1999 and 1996). To further explore the role of PKR in regulating immune response, we examined whether overexpression of PKR results in the induction of IFN and related cytokines. Promonocytic U937 and lymphoblastoid Namalwa cells were transfected with control and PKR expression plasmids to generate stable transfectants. By RT-PCR and kinase assays, we showed that cells transfected with PKR plasmids have higher levels of PKR expression and activity, compared to the corresponding wild-type cells. Following treatment of these cells with poly(I):poly(C), the cells were examined for cell viability and cytokine levels by FACS analysis and ELISA. We showed that the PKR-overexpressing cells were more sensitive to poly(I):poly(C) treatment and underwent apoptotic cell death earlier than controls. However, these cells showed high levels of induced expression of IFN-α and -β, as well as other proinflammatory cytokines including IL-6, IL-8 and TNF-β. Treatment of these PKR-overexpressing cells with 2-aminopurine, an inhibitor of PKR, resulted in lower levels of expression of the fore-mentioned cytokines, indicating a key role for PKR in the induction process. Thus, it is paradoxical that PKR, as a translation inhibitor, is critical in upregulating immune protein expression. These experiments indicate an essential role for PKR in regulating antiviral activities and inflammatory responses. This dsRNA-PKR system may be used as a paradigm to study the interaction of virus with the host cells and the mechanisms of cytokine gene regulation.

16004

SIGNAL TRANSDUCTION PATHWAYS INVOLVED IN APOPTOSIS INDUCTION BY THE IFN-INDUCED PROTEIN PKR

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The double stranded (ds) RNA-dependent protein kinase (PKR) has been shown to mediate apoptosis induced by different stimuli. By using recombinant vaccinia virus (VV) expressing PKR under regulation, we have characterized the role of PKR targets in apoptosis induction. We have shown that apoptosis induction by PKR involves eIF-2α phosphorylation and activation of the transcription factor NF-κB. Activation of NF-κB by PKR involves the IKK complex and is dependent of PKR kinase activity. In addition, PKR induced apoptosis involves FADD activation of caspase 8, based on four experimental findings: upregulation of caspase 8 activity during PKR-induced apoptosis, blocking of PKR-induced apoptosis by the use of a chemical inhibitor of caspase 8, and inhibition of PKR-induced apoptosis by expression of both a FADD dominant negative or a viral FLIP molecule. Significantly, despite that PKR expresion induces upregulation of Fas mRNA expression, neither the Fas receptor-ligand nor TNFα-TNFR1 pathways are needed for PKR-induced apoptosis. The identification of molecules acting upstream ofthe IKK complex on PKR-mediated NF-κB activation is of critical importance in signalling, and this is under investigation. Overall, a comprehensive explanation is emerging to explain the wide biological effects of PKR.

16005

THE CATALYTIC ACTIVITY OF PKR IS NEEDED FOR NF-κB ACTIVATION BY THIS IFN-INDUCED KINASE

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Besides its known role as a translational controlling factor, the double stranded RNA-dependent protein kinase (PKR) is a key transcriptional regulator exerting antiviral and antitumoral activities. By expressing PKR using an IPTG-inducible vaccinia virus (VV) recombinant we have shown that induction of NF-κB by PKR is channeled through activation of the IKK complex and is involved in apoptosis commitment. To gain insights into the mechanism of activation of NF-κB by PKR, we have used a collection of VV recombinants expressing different PKR deletion mutants in $PKR^{0/0}$ cells. We showed that only PKR forms conserving catalytic activity are able to activate NF-κB. An inactive PKR mutant (K296R) is unable to induce NF-κB activation under a wide range of concentrations, as defined by Western blot, IKK activity, EMSA and NF-κB transactivation assays. However, PKR mutants unable to activate NF-κB retain their ability to associate with the IKK complex, as shown by immunoprecipitation analysis. We concluded that catalytic activity of PKR and not only physical interaction with the IKK complex is needed for activation of the transcription factor NF-κB.

16002

NOVEL INTERFERON STIMULATED GENES (ISGs) POTENTLY INDUCED BY IFN-β IN WM9 MELANOMA CELLS

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Protein products of ISGs must underlie changes in cellular function leading to clinical activity of IFNs. We postulate that gene products, not yet identified or only minimally studied functionally, may be important in resulting therapeutic responses. The mRNA profile for ISGs induced by IFN-α2, IFN-β, and IFN-γ has been assessed in the human fibrosarcoma cell, HT1080, by using oligonucleotide arrays with probe sets corresponding to more than 5700 human genes. IFN-β was particularly effective in inducing gene expression--more than 30 genes 3x or more that were not induced by IFN-α2. We have now extended these studies to a melanoma cell line, WM9, but limited study to IFN-α2 or IFN-β. Using our published criteria, over 105 genes were identified as induced. More than a dozen genes were induced 10x or more but only one to a quantitatively greater extent by IFN-α2 than IFN-β (a p44 protein); more than 15 genes were increased by IFN-β but not by IFN-α2. We have confirmed 5 of these genes, not previously known to be ISGs, by Northern blot, RNase protection assay (RPA), or RT-PCR: lectins (galectin 9), apoptosis and/or growth pathways (TRAIL, XAF-1, MGSA), and immune cell interactions (K12). We have also confirmed in WM9 cells, as new ISGs, BST2, phospholipid scramblase, and Ro autoantigen, first identified in oligonucleotide array in HT1080 cells. Samples from 3 time points (6, 18, and 36 hrs) were pooled for array analysis, Northern blot or RPA was used to identify differences at early and late time points. In a third oligonucleotide array study, we have confirmed induction of some ISGs as early as 1 hr. These findings suggest that customized ISG arrays may be a basis for correlating gene induction with therapeutic response.

16007

A TRUNCATED FORM OF RNASE L ACCUMULATES IN PBMC OF CHRONIQUE FATIGUE SYNDROME PATIENTS

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A 37kDa 2-5A binding polypeptide accumulating in PBMC extracts from CFS patients is evaluated as a biochemical marker for the disease (De Meirleir et al, 2000).
This 37kDa 2-5A binding polypeptide has a nuclease activity and is therefore considered as a low molecular weight and possibly dysregulated form of RNase L ( ). This sheds new light on the physiopathology of the disease since RNase L is capable of destabilizing mRNAs controlling terminal myoblast differentiation (Bisbal et al, in press) or mitochondrial activity .
Incubation of CFS PBMC extracts without protease inhibitors leads to fast disappearance of 80kDa RNase L and to accumulation of the low molecular form in keeping with a possible involvement of proteases. Increased proteolytic activity in CFS PBMC as a major mechanism for the accumulation of the LMW form of RNase L has now been confirmed. Indeed, complementation of 2-5A labeled recombinant HuRNase L with CFS PBMC extracts leads to the accumulation of 37kDa polypeptide. This cleavage product and the LMW form of RNase L isolated form CFS patients PBMC give rise to identical sets of 2-5A labeled peptides upon fingerprint analysis.
Intriguingly the 2-5A binding and catalytic domains of RNase L cannot be associated in a single proteolytic cleavage fragment according to published structure-function analysis data .
The identity and the origin of the proteases involved in the processing of RNase L in CFS patients PBMC is currently investigated.

De Meirleir et al. Am. J. Med. 108 (2000) 99-104

Cytokine-binding proteins

17004

REGULATION OF CYTOKINE ACTIVITIES AND HALF-LIFE BY THEIR BINDING PROTEINS

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Body fluids contain relatively high levels of specific binding proteins, which form reversible complexes with most known cytokines, hormones and growth factors. Most cytokine binding proteins are translation products of mRNA splice variants, corresponding to the extra-cellular ligand-binding domain of the cell surface cytokine receptor. Alternatively, they are shed by proteolysis of the cell surface receptor. The splicing pattern, as well as the protein cleavage sites, are evolutionary conserved, indicating an important physiological role of these binding proteins.
With very few exceptions, high concentrations of cytokine binding proteins may inhibit the cytokine activity by competing for ligand binding with their cell surface homologues. However, this is not necessarily their physiological role. The soluble Type I IFN receptor (IFNAR2a) is translated from a specific mRNA splice variant. Its plasma level is much higher than that of the Type I IFNs, indicating that plasma IFNs exist mostly as complexes with IFNAR2a. Binding studies using the BIAcore system show that the IFN-IFNAR2a complex is reversible. Since the affinity of the cell surface IFN receptor for IFN is ~10-fold higher than that of IFNAR2a, a possible role of the later as a carrier protein was investigated. Co-administration of IFN-beta with IFNAR2a to monkeys considerably elongated its plasma half-life, elevating the level of biologically active IFN-beta up to 90-fold. Similarly, IFNAR2a elongated the survival of SCID mice challenged with Daudi cells when co-administered with human IFN-alpha2. These results suggest that IFNAR2a may serve as a carrier protein, whose function is to elongate and enhance the action of Type I IFNs in vivo. By analogy, it is likely that most other cytokine binding proteins having similar characteristics serve as carrier proteins rather than cytokine inhibitors.

17003

IL-18 BINDING PROTEIN IN HEALTH AND DISEASE

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IL-18, an IFN-γ inducing factor, is a Th1 cytokine, structurally related to the IL-1 family. We purified from normal human urine, a constitutively expressed IL-18 binding protein (IL-18BP) showing no homology to IL-18 cell surface receptors. It binds IL-18 with a very high affinity (Kd= 400 pM) and exerts a very slow off rate. Thus, IL-18BP probably functions as an inhibitor rather than a stabilizer of its ligand. Indeed, IL-18BP abolished the induction of IFN-γ and IL-8, and the activation of NF-κb in vitro, and abrogated LPS induced IFN-γ in vivo. Four alternatively spliced transcripts were identified. Only isoforms a and c of IL-18BP bound IL-18. IL-18BP does not bind the immature pro IL-18 or IL-1β. Sandwich ELISAs to IL-18BPa were developed. IL-18BPc cross reacted with IL-18BPa while isoforms b and d were not recognised. The ELISAs were not interfered by IL-18 at the highest levels found in pathological situations. IL-18BPa levels in healthy individuals sera ranged from 0.5 to 4.5 ng/ml with an average of 2 ng/ml. No gender or age effect was observed. Data on the levels of IL-18BPa in various pathological situations will be presented. For example in patients with sepsis and acute renal failure the IL-18BPa levels are very much elevated but do not correlate with creatinine levels, therefore probably represent increased production rather than retention.

17002

TIP-B1, A Novel Tumor Necrosis Factor-α Inhibitory Protein

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A novel tumor necrosis factor-α (TNF) inhibitory protein, TIP-B1, has been identified, purified and characterized from cytosolic extracts of TNF-treated human fibroblasts, and a partial TIP-B1 cDNA clone has been obtained (Berleth et al., *Cancer Res.* 59: 5497-5506, 1999; *Intl. J Immunopharm.*, in press, 2000). The ~27 kDa pI~4.5 TIP-B1 protein is unique based on both the sequence of three internal peptides (comprising 51 amino acids), and the nucleotide sequence of the corresponding cDNA clone. TNF-sensitive cells, when exposed to TIP-B1 prior to the addition of TNF, are completely protected from TNF-induced lysis. Thus, the addition of TIP-B1 to cells in culture for ≥4h effectively makes them resistant to concentrations of TNF that would otherwise cause induction of cytolysis (including apoptosis). Evidence has been obtained that TIP-B1 is not a soluble TNF receptor, nor an anti-TNF antibody, nor a protease which degrades TNF, yet TIP-B1 functions when added exogenously to cells. These data indicate that TIP-B1 is not one of the other proteins previously reported to be involved in resistance to TNF. TIP-B1 has been shown to be constitutively expressed in human fibroblasts. Low, non-toxic concentrations of TNF, which renders human fibroblasts protected from cytotoxic concentrations of TNF, induces increased expression of TIP-B1 in those cells. It has been shown in cells cultured in low concentrations of TNF, that the protection afforded them is linked to changes in cytosolic and membrane TIP-B1 levels. The fact that incubation of the novel TIP-B1 with TNF-sensitive cells protects them from TNF-induced cell death, including TNF-mediated apoptosis, suggests that TIP-B1 has unique potential for basic studies of TNF regulation and for therapeutic applications. [Funded in part by grants CA16056 & CA09072 (USADHHS, NIH, NCI)].

17005

Treatment hIL18 binding protein ameliorates experimental colitis

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IL18 is a pro-inflammatory cytokine that plays an important role in the immune regulation in Crohn's disease. It was shown to be upregulated in Crohn's disease and during TNBS colitis. IL18 binding protein (IL18BP) is a soluble decoy receptor for IL18, which can effectively antagonise the biological effects of IL18.
In this study experimental colitis was induced by intra-rectal administration of TNBS. Mice were treated daily with hIL18 binding protein (hIL18BP). The weight loss induced by the colitis was significantly less in mice treated with hIL18BP compared to control treated TNBS mice. In addition, other inflammatory parameters such as colon weight were significantly decreased in treated mice. The histological score of hIL18BP treated mice with colitis was greatly diminished compared to control treated mice with colitis. Less inflammatory cells invaded the area of inflammation and ulcerations were absent in treated mice. The IFNγ and TNFα production of caudal lymph node and spleen cells stimulated in vitro with CD3/CD28 showed no difference between the two different treatment groups. However, TNFα levels in colon homogenates of TNBS mice treated with hIL18BP were significantly reduced compared to TNBS mice treated with saline. The effect of hIL18BP seems to be mediated by a direct effect on the IL18-induced TNFα production. Importantly, this effect is independent of IFNγ. Hence, treatment with hIL18BP reduced disease in experimental colitis and therefore has potential therapeutic properties in inflammatory bowel disease

17006

SECRETION OF RECOMBINANT CYTOKINES VIA THE CHAPERONE/USHER PATHWAY IN *ESCHERICHIA COLI*

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Capsule F1 antigen (Caf1) of *Yersinia pestis* is assembled via the chaperone (Caf1M)/ usher (Caf1A) pathway. We investigated secretion of entire heterologous proteins fused to Caf1 in the presence of Caf1M or Caf1M/Caf1A in *Escherichia coli*. Despite correct processing, a chimeric protein composed of modified Caf1 signal peptide, mature human interleukin-1β (hIL-1β), and mature Caf1 (CIC) remained insoluble. Co-expression of CIC with functional Caf1M led to accumulation of soluble heterogeneous Caf1M-$[CIC]_n$ complexes in the periplasm. Soluble CIC reacted with monoclonal antibody (mAb) directed against structural epitopes of hIL-1β. The results indicate that Caf1M induced release of processed CIC from the inner membrane promotes folding of the hIL-1β domain. Similar results were obtained with fusion of Caf1 to a receptor antagonist hIL-1 (hIL-1ra) or to granulocyte-macrophage colony-stimulating factor (hGM-CSF). Following co-expression of CIC with both Caf1M and Caf1A outer membrane protein, CIC could be detected on the cell surface of *E. coli*. These results demonstrate for the first time a flexibility of the chaperone/usher secretion pathway in the transport of subunits with heterogeneous N-terminal domains. This represents a novel means for the delivery of correctly folded heterologous proteins to the periplasm and cell surface as either polymers or cleavable domains.

17001

ROLE OF HUMAN IL-1β IN THE STIMULATION OF PROLIFERATION OF BACTERIAL CELLS EXPRESSING CAPSULAR SUBUNIT PROTEIN CAF1 OF *Y.PESTIS*

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Previously we have shown that hIL-1β specifically binds with *E. coli* cells expressing capsular Caf1 protein of *Y. pestis*. At the surface of the transformed cells is formed the capsule, consisting from the large oligomers of Caf1 subunit. Capsular subunit protein Caf1 of *Y.pestis* was found to compete with radiolabelled hIL-1β for binding with bacterial cells. To investigate more precisely the role of hIL-1β in the pathogenesis of bubonic plague, we examined the ability of hIl-1β to bind with recombinant Caf1 protein *in vitro*. The purified Caf1 protein is in a polymerized form (MW more than 10^6). It consists of many oligomers with even number of subunit. The smallest oligomer is dimer with molecular mass 31 kDa. The ability of hILs-1 to bind with different oligomers of recombinant Caf1 protein was tested. Dimer of Caf1 (formed after boiling of polymeric form of protein), but not the polymer Caf1, was able to bind with hIL-1β, that was evidenced by ELISA with purified recombinant proteins. The *in vitro* results have shown also that IL-1β prevents polymerization of Caf1 dimer. The *in vivo* results showed that hIL-1β has effect on the proliferation of *E. coli* cells expressing Caf1 protein and on the synthesis and secretion of Caf1 protein. Recombinant hIL-1β stimulated the proliferation of *E. coli* cells carrying *f1* operon *Y. pestis* and decreased the synthesis and secretion of Caf1 protein. These results suggest that heterooligomerization of IL-1β with Caf1 dimer at early stages of capsule formation can play the important role in pathogenesis of a bubonic plague.

17009

REGULATION OF INTERFERON RESPONSES

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The type I and II interferons (IFNs) are examples of cytokines which can have a positive effect on host defense against a range of diseases, yet if their responses are excessive or inappropriate, could have severe adverse effects. The IFNs protect cells from viral infection, possess antitumour cells and can regulate most effector cells of the immune system. However, IFN therapy has often severe dose-limiting "toxicity" which includes nausea, malaise, headaches, leukopenia and sometimes autoimmune reactions. It is therefore important that the effects of these endogenous IFNs are fine-tuned to provide maximum benefit with minimum adverse effects. Both positive and negative regulation of IFN responses occur outside and inside the cell. The recently identified Suppressors of Cytokine Signaling proteins (particularly SOCS1 and SOCS3) are important negative regulators of IFN signaling. Mice with a null mutation in SOCS1 die neonatally from hypersensitivity to IFNγ and can be "rescued" by crossing with mice deficient in the IFNγ gene. These mice are also hypersensitive to IFNα and indeed show increased resistance to viral infection and amplified molecular signaling in response to type I IFNs. The type I IFN signal can also be modulated in the extracellular compartment by a soluble receptor isoform which is differentially expressed by certain tissues, is present in serum and has the dual ability to either block or present IFNα and β for cell signaling. The soluble receptor might therefore be an important regulator of responses to endogenous and exogenous IFNs. Understanding these various modes of negative regulation of IFN signaling will be important to elucidate the role of IFNs in disease processes and in improving and predicting clinical responses.

17007

A Role for sIL-6R and Synovial Fibroblasts in Leukocyte Recruitment in Rheumatoid Arthritis.

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IL-6 and its soluble receptor (sIL-6R) are elevated in serum and synovial fluids of Rheumatoid Arthritis (RA) patients. The contribution of the [sIL6R/IL-6] complex to RA has so far been indistinguishable from that of IL-6 alone. Two isoforms of sIL-6R exist, one is released as a result of differential mRNA splicing (DS-sIL-6R) and the other is shed from the cell membrane by proteolytic cleavage (PC-sIL-6R). The relative abundance of both isoforms was investigated in serum of both healthy and RA individuals. Quantification of each form was assessed by ELISA. Total sIL-6R levels were found to be ~2-3 fold higher in RA patients than in normal individuals. These increases were not due to release of DS-sIL-6R suggesting augmentation of PC-sIL-6R. This differential pattern of release was also confirmed by Western blot analysis of partially purified sIL-6R isolated from patient's sera. This implies that release of PC-sIL-6R is specifically activated during inflammatory crisis. During RA, synovial fibroblasts undergo hyperplasia and the [sIL-6R/IL-6] complex can induce synoviocyte proliferation *in vitro*, while leukocyte infiltration into arthritic joints can be correlated to an increase in sIL-6R in the synovial fluid. To determine whether synovial fibroblasts are likely to contribute to regulation of leukocyte recruitment, we examined whether human IL-6, sIL-6R or a chimeric [sIL-6R/IL-6] fusion protein (Hyper IL-6 [HIL-6]) could affect expression of MCP-1, IL-8 and RANTES. Human synovial fibroblasts were obtained from synovial membranes of RA patients and the affect of sIL-6R and IL-6 on chemokine expression investigated by ELISA. Neither IL-6 nor PC-sIL-6R alone had any significant effect on MCP-1 production in any of the primary cell cultures, however, HIL-6 (10-100 ng/ml) induced a time and dose dependent increase in MCP-1 levels (IL-1β & TNF also induced MCP-1) resulting in ~5-fold induction. In contrast to IL-1β and to a lesser extent TNFα, IL-6, PC-sIL-6R (or in combination) and HIL-6 did not promote IL-8 or RANTES production. These data suggest that elevated levels of PC-sIL-6R in RA patients could have a profound influence on the synovial environment and as a result, expedite the influx of monocytes and lymphocytes into the arthritic joint.

17008

A novel soluble tumor necrosis factor receptor, CrmE, encoded by poxviruses.

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Cytokines and chemokines play a critical role in both the innate and acquired immune responses and constitute prime targets for pathogen sabotage. Molecular mimicry of cytokines and cytokine receptors is a mechanism encoded by large DNA viruses to modulate the host immune response. Three tumor necrosis factor receptors (TNFRs) have been identified in the poxvirus cowpox virus. Here we report the identification and characterization of a fourth distinct soluble TNFR, named cytokine response modifier E (CrmE), encoded by cowpox virus. The *crmE* gene has been sequenced in strains of the orthopoxviruses cowpox, ectromelia and camelpox viruses, and was found to be active in cowpox virus. *crmE* is expressed as a secreted 18 kDa protein with TNF binding activity. CrmE was produced in the baculovirus and vaccinia virus expression systems and was shown to bind human, mouse and rat TNF, but not human lymphotoxin α or several other ligands of the TNF superfamily. However, CrmE only protects cells from the human TNF cytolitic activity. CrmE is a new member of the TNFR superfamily which is expressed as a soluble molecule that blocks the binding of TNF to high affinity TNFRs on the cell surface. The remarkable finding of a fourth poxvirus-encoded TNFR suggests that modulation of TNF activity is complex. This novel viral immune evasion mechanism provides insights into virus-host interactions and new strategies of immune modulation.

Immunosuppressive cytokines

18006

SMAD3 – A MAJOR PLAYER IN SIGNAL TRANSDUCTION PATHWAYS LEADING TO FIBROGENESIS?

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Transforming growth factor-β, TGF-β, plays a central role in fibrosis, contributing both to the influx and activation of inflammatory cells, as well as to activation of fibroblasts to elaborate extracellular matrix. In the past few years, new insight has been gained into signal transduction pathways downstream of the TGF-β receptor serine-threonine kinases with the identification of a family of evolutionarily conserved Smad proteins. Two receptor-activated Smad proteins, Smad2 and Smad3, are phosphorylated by the activated TGF-β type I receptor kinase, and together with Smad4 participate in transcriptional complexes to control expression of target genes. In wound healing studies of mice null for Smad3, we show that loss of this key signaling intermediate interferes with the chemotaxis of inflammatory cells to TGF-β as well as with their ability to autoinduce TGF-β. Moreover, studies with mouse embryo fibroblasts null for Smad3 show that TGF-β-dependent induction of c-Jun and c-Fos, important in induction of collagen as well as in autoinduction of TGF-β, is mediated by Smad3. Based on these observations, we hypothesize that loss of Smad3 will confer resistance to fibrosis and result in reduced inflammatory cell infiltrates, reduced autoinduction of TGF-β, important to sustain the process, and reduced elaboration of collagen. Preliminary observations in a model of radiation-induced fibrosis confirm this hypothesis and suggest that inhibitors of Smad3 might have clinical application both to improve wound healing and to reduce fibrosis.

18005

INHIBITION OF PROTEOSOMAL Z-LEU-LEU-LEU-AMC HYDROLYSIS: A NOVEL MECHANISM OF GROWTH INHIBITION BY TRANSFORMING GROWTH FACTOR β (TGFβ).

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The mechanisms mediating growth inhibition by TGFβ remain obscure. TGFβ increases $p27^{KIP1}$, a potent regulator of cell cycle progression, in many cell types. Our **AIM** was to test the **HYPOTHESIS** that TGFβ inhibits growth by decreased proteosomal degradation of $p27^{KIP1}$. **METHODS**: Specific proteosome-associated hydrolytic activities were assayed in human biliary epithelial cells using the fluorogenic substrates Z-Leu-Leu-Leu-AMC (LLL), Suc-Leu-Leu-Val-Tyr-AMC (LLVY), or Z-Leu-Leu-Glu-AMC (LLE). Expression of $p27^{KIP1}$, $p21^{WAF1}$, $p16^{INK4A}$ and HSP90 was assessed by immunoblotting. Z-Leu-Leu-Leu-aldehyde (MG132) was used to inhibit proteosomal activity. **RESULTS**: TGFβ inhibited cell growth in a concentration-dependent manner (59±2% inhibition with 1 ng/ml TGFβ after 24 hrs), but did not induce significant apoptosis. TGFβ markedly decreased hydrolysis of LLL (41±15% and 93±1% inhibition after 24 hrs with 0.1 and 1 ng/ml TGFβ respectively). However, TGFβ (0.1-10 ng/ml) did not inhibit VLLY or LLE hydrolysis. Incubation with 1 ng/ml TGFβ increased $p27^{KIP1}$ expression to 129±9% and 143±12% of controls at 6 and 24 hrs respectively, but did not alter expression of $p21^{WAF1}$ or $p16^{INK4A}$. MG132 inhibited LLL hydrolysis in cell extracts (IC_{50}: 24nM). Incubation with 50nM MG132 for 24 hrs increased $p27^{KIP1}$ expression to 144±13% of controls. Furthermore, MG132 decreased cell proliferation in a concentration dependent manner. Incubation with TGFβ 0.1ng/ml, did not increase expression of HSP90, an endogenous inhibitor of LLL activity. In **CONCLUSION**, these novel data indicate that TGFβ decreases proteosomal LLL hydrolysis via an HSP90 independent mechanism with subsequent increase in $p27^{KIP1}$ levels, and inhibition of growth.

18003

EFFECT OF INTERLEUKIN-10 ON *COXIELLA BURNETII* REPLICATION IN HUMAN MONOCYTES

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Coxiella burnetii, an obligate intracellular microorganism that infects myeloid cells, is the agent of Q fever. Q fever endocarditis is characterized by defective cell-mediated immunity and the overproduction of IL-10 and TGFβ1 by human monocytes. We hypothesize that the intracellular fate of *C. burnetii* depends on immunoregulatory cytokines including IL-10, TGFβ1 or IL-4. In the absence of cytokines, *C. burnetii* survived but did not replicate in human monocytes. When IL-10 was added to monocytes before infection with *C. burnetii*, bacterial uptake and replication (measured during 15 days) were significantly increased as compared to untreated cells. IL-4 was less potent than IL-10 and TGFβ1 had no effect. Cytokine-mediated replication of *C. burnetii* did not depend on the level of bacterial uptake and was observed in infected cells post-treated by cytokines. The mechanism of *C. burnetii* replication induced by IL-10 involves TNF. TNF release stimulated by *C. burnetii* was completely inhibited by IL-10. Neutralizing anti-TNF Ab mimicked IL-10 and recombinant TNF did not allow bacterial replication. The difference between IL-10 and IL-4 is related to the upregulation of TNF-RII release by IL-10, whereas IL-4 had not effect on TNF-RII release. On the other hand, *C. burnetii* replication was observed in patients with chronic Q fever but not in patients with acute Q fever. This increase was prevented by neutralizing anti-IL-10 Ab. As monocytes from patients with chronic Q fever overproduced IL-10, the defective bacterial killing is likely related to the presence of endogenous IL-10. These results suggest that IL-10 enables monocytes to support *C. burnetii* replication and favor the development of chronic Q fever.

18001

STAT3 IS A MOLECULAR TARGET FOR ESTROGEN RECEPTOR INHIBITION OF THE IL-6 SIGNALLING PATHWAY IN HUMAN MULTIPLE MYELOMA CELLS

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Multiple myeloma (MM), a clonal B-cell malignancy, is characterized by the expansion of malignant plasma cells in the bone marrow and by the presence of osteolytic lesions. Interleukin-6(IL-6) has been identified as a major cytokine involved in the emergence of the tumor clone and in tumor-associated toxicity in MM patients. Interestingly, most human MM cell lines and patient MM cells express estrogen receptor α (ERα). Little is known about why they express high levels ERα or the biological function and molecular consequences of ER activation on MM cell biology. We chose two novel human IL-6-dependent myeloma cell lines, KAS-6/1 and ANBI.6 from patients with aggressive disease, as model systems. These cell lines are all IL-6 responsive and therefore are phenotypically representative of freshly isolated tumor cells as well as expressing high levels of ERα. The ER-agonist, 17-β-estradiol completely abolished IL-6-inducible [^{3}H]-thymidine incorporation in a dose dependent manner. By contrast, the ER-antagonist, 17-α-estradiol at the same concentration did not inhibit IL-6-mediated cell proliferation. These data suggested ER downregulated the IL-6-induced cell growth signal in MM cells. To identify the signaling molecule responsible for the estrogen inhibition of myeloma cell responses to IL-6, we investigated the effect of ER ligands on the IL-6 regulated JAK/Stat3 signalling pathway. We have found estrogen markedly inhibited Stat3 DNA binding and transactivation, but failed to affect tyrosine phosphorylation of JAK2 and Stat3. ER was shown to form a complex with Stat3 suggesting a possible mechanism of Stat3 inhibition requiring the physical association of ER and Stat3. Several cell cycle genes were inhibited including p27 and Rb, but not E2F or CDK6. These data directly demonstrate Stat3 is a molecular participant in ER inhibition of the IL-6 signalling pathway in human MM cells and has implications for the potential use of estrogenic ligands in the treatment of MM or other tumors where IL-6 may have an autocrine or paracrine role.

18004

APC DERIVED CYTOKINES BUT NOT T-CELL DERIVED CYTOKINES ARE UPREGULATED IN PATIENTS ON CHRONIC HEMODIALYSIS

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Patients on chronic hemodialysis (CHID) suffer from general immune incompetence, resulting in a high incidence of infections and malignancies. This immune incompetence could be the result of imbalanced cytokine systems. To study whether these cytokine systems are affected in CHID patients (N=15) we measured mRNA expression levels of APC derived cytokines (IL-10, IL-15, TNFα) and of T-cell derived cytokines (IL-2, IFNγ) in peripheral blood mononuclear cells (PBMC) by competitive template RT-PCR. Samples from healthy controls (N=11) served as control. For the APC derived cytokines we found a significant higher mRNA expression level in PBMC of CHID than PBMC of healthy controls. The median TNFα/keratin ratio in CHID was 47,541 (range 2,734-136,279) vs. 528 (range 11-28,855) in controls, p<0.001. For IL-15, the median expression for IL-15/keratin ratio in hemodialysis patients was 5 (range 2-16) and for controls 2 (range 0.2-9), p=0.025. In contrast, the mRNA expression level of IFNγ, IL-2 and IL-10 were comparable between CHID patients and controls. The median IFNγ/keratin ratio was 1 (range 0.2-4) vs. 0.5 (range 0.1-2) and median ratio for IL-10 0.01 (range 0.01 -26,360) vs. controls 0.01 (range 0.01-9,520). There was no difference in the proportion positive IL-2 samples between CHID patients and controls.
In conclusion, we found high expression level of APC-derived cytokines (IL-15, TNFα). However this did not result in upregulation of T-cell derived cytokines (IL-2, IFNγ). Their dysbalanced cytokine systems may contribute to the immune incompetence in patients on chronic hemodialysis.

18002

THE MECHANISM OF ACTION OF MYCOPHENOLIC ACID AND METHOTREXATE.

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Introduction: Methotrexate (MTX) and mycophenolic acid (MPA) are currently applied in the clinic as immunosuppressants. MTX is widely used in the treatment of RA. MPA is used in transplantation and is now experimentally used in RA with promising results. The precise mechanism of action is still debated. It is known that the drugs, although in a different way, inhibit the *de novo* synthesis of DNA and RNA.
Aim: We would like to understand the working mechanism of MPA and MTX.
Methods: Measurement of cytokine production by ELISA after short cell cultures. Analysis of the expression of activation markers by FACS.
Results: We have found that both drugs inhibit the production of IL-2, IL-4, IL-13, IFN-γ, TNF-α and GM-CSF in CD3/CD28 stimulated whole blood cultures. However there are differences. In contrast to MPA, MTX does not inhibit the production of IL-8. The inhibition of MPA is evident at day 1, whereas the inhibition by MTX can be observed from day 3 on. Furthermore MTX has no effect on SAC or LPS induced cytokine production whereas MPA lowers this cytokine production. When human peripheral blood-derived mononuclear cells or T cells are activated in the presence of MTX, they die by apoptosis. Resting cells are not affected. The mechanism of MPA is different. Cycling T cells become apoptotic after addition of MPA. However when resting T cells are activated in the presence of MPA, some activation markers such as CD69 and CD25 are upregulated, but the cells stay small and do not enter S phase. This inhibition by MPA is reversible, even after fourteen days of culture.
Conclusions: MPA works more rapidly than MTX and independent of proliferation. MTX leads to irreversible elimination of the activated cells by apoptosis, whereas the MPA reversibly prevents proliferation of resting cells.

18009

TGF-β REGULATES T CELL ADHESION TO AND MIGRATION THROUGH FIBRONECTIN: ROLE OF PYK2

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TGF-β plays an important role in the regulation of leukocyte maturation, proliferation and migration. In the present work, we explored the effects of short-term (1h) treatment with TGF-β_1 on T lymphocyte adhesion to and migration through fibronectin (FN), which is a prototypic cell-adhesive extracellular matrix protein. We found that TGF-β alone elevated T cell adhesion to FN, whereas the combined action of TGF-β and IL-2 induced a decreased level of adhesion as compared to TGF-β and IL-2 alone. When T cells were pretreated with TGF-β, then washed and exposed to IL-2 adhesion remained normal. However, when the cells were pre-incubated with IL-2 prior to washing and TGF-β exposure, TGF-β failed to induce adhesion. T cells pretreated with TGF-β demonstrated decreased migration on SDF-1α gradients in experiments using the Transwell system. Moreover, TGF-β placed into the lower chamber together with SDF-1α diminished T lymphocyte migration as well. Studies on the mechanisms underlying these effects indicated that short-term incubation with TGF-β did not modify cell surface expression of β_1 integrins or CXCR4 chemokine receptor. However, TGF-β increased phosphorylation of the focal adhesion kinase family member Pyk2, and this effect was more pronounced when the cells were incubated on FN. In parallel, TGF-β decreased Pyk2 phosphorylation induced by IL-2. Pyk2 phosphorylation induced by TGF-β on FN was partially inhibited by blocking integrins or TGF-βRII. We conclude that TGF-β regulates adhesion and migration of T cells, and this effect is probably mediated by Pyk2.

18010

A CHINESE HERBAL MEDICINE, FU-LING, REGULATES THE PRODUCTION OF INTERLEUKIN-10 FROM MURINE SPLEEN CELLS

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Fu-Ling, the sclederma of *Poria cocos* (Schw.) Wolf, has long been used as a sedative and diuretic in traditional Chinese herbal medicine. Our previous studies suggested that Fu-Ling extract significantly regulated IL-1β, IL-6, TNF-α, TGF-β and GM-CSF production *in vitro*. In this report, the regulatory effect of Fu-Ling on interleukin-10 (IL-10) was investigated. Mice were i.p. injected with various doses of Fu-Ling daily for three consecutive days. The spleen cells were then isolated and *in vitro* cultured for three days with Con A. At the end of culture period, the IL-10 secretion and the level of IL-10 mRNA expression were measured. Result indicated that spleen cells isolated from the mice treated with Fu-Ling significantly increased IL-10 secretion and mRNA expression in a dose-dependent manner. Since IL-10 is a potent differentiation factor of B-lymphocytes, the possible role of Fu-Ling in regulation of immunoglobulin (Ig) production was studied *in vivo*. Results indicated that spleen cells isolated from the mice treated with Fu-Ling did significantly increased IgG and IgA secretion but no effect on IgM secretion. Thus, Fu-Ling might induce the differentiation of B-lymphocytes *via* stimulating the IL-10 production. Our preliminary data suggested that a steroid-type compound isolated from Fu-Ling extract might be the biologically active ingredient.

18007

PRIMING AND INHIBITING EFFECTS OF INTERLEUKIN-10 (IL-10) ON LPS-INDUCED MONOCYTE ACTIVATION

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IL-10 is known as an anti-inflammatory cytokine. However, we reported *in vitro* experiments where IL-10 can have an opposite effect and prime human leukocytes (*Intern. Immunol. 1999, 11, 689*). We observed that in whole blood, pretraitment of human peripheral blood mononuclear cells (PBMC) with IL-10 increased their capacity to produce TNF in response to LPS. This phenomenon is reproduced when the PBMC are pretreated with IL-10 on Teflon® which prevents adherence of monocytes, but not when the cells are pretreated on plastic. In the latter case, IL-10 inhibited LPS-induced TNF production in agreement with previous reports. We further analyzed the influence of adherence on IL-10 signaling. We studied the activation of STAT3 and the expression of the protein CIS, a member of the suppressor of cytokine signaling (SOCS) family. Both on Teflon® and plastic, IL-10 induced STAT3 activation (*i.e.* phosphorylation), but the activation was significantly more pronounced on Teflon® than on plastic. Similarly, the expression of CIS was more important in PBMC treated with IL-10 on Teflon® as compared to plastic. The activation of STAT1 protein is under investigation. We have also studied the effect of IL-10 on the adhesion molecules (CD11a, CD11b, CD62L) and on the cells surface markers (CD14, CD40, HLA-DR). IL-10 appears to decrease the expression of CD11a, CD40, HLA-DR and CD11b on PBMC in both conditions (Teflon® and plastic). In contrast, IL-10 has an opposite effect on the expression of CD62L between plastic and Teflon® conditions. CD62L is upregulated by IL-10 on Teflon® while its expression is down-regulated on plastic. The paradoxical effect of IL-10 could not be explained by the up-regulation of CD14, because its expression was increased in both Teflon® and plastic conditions. The effect of IL-10 on Toll-like receptors (TLR)-2 and -4, which have been shown to be the co-receptors of LPS, is currently under investigation. This study shows the critical influence of adhesion of monocytes/macrophages on the properties of IL-10 on these cells.

18008

IFNα ALTERS IL-4 RECEPTOR EXPRESSION AND IL-4 REGULATION OF TNFα AND IL-10 PRODUCTION BY HUMAN MONOCYTES

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Interleukin-4 (IL-4) can suppress LPS-induced IL-1β, IL-12, TNFα and IL-10 production by monocytes. In contrast, IL-4 can suppress LPS-induced IL-1β and IL-12, but not TNFα or IL-10 production by inflammatory macrophages *ex vivo* (i.e. synovial fluid mononuclear cells, SFMC) and monocyte-derived macrophages (MDMac). Classically, the receptor for IL-4 consists of the IL-4 binding *IL-4Rα* chain and the IL-2Rγ (γc) chain. With monocyte culture the surface expression of γc is reduced and ^{125}I-IL-4 bind three IL-4Rα chains for every γc chain. This relative reduction in γc correlates with decreased STAT6 activation by IL-4 and IL-4 dysregulation of TNFα and IL-10 production IFNα and IL-10 production increase with monocyte culture. Monocytes cultured with IFNα, but not IL-10, for 24-48 h show the same IL-4 response profile as SFMC and MDMac. That is, IFNα-treated monocytes have reduced γc expression and IL-4 can regulate LPS-induced IL-1β but not TNFα production. IFNα levels as high as 3 ng/ml have been detected in synovial fluids taken from inflamed joints. These results suggest that IFNα is the molecule responsible, by its effects on γc, for selective IL-4 regulation of inflammatory mediators produced by macrophages.

The renaissance of IFN-β including its effect on MS and EAE

00005

TREATMENT OF MULTIPLE SCLEROSIS PATIENTS WITH INTERFERON-β PRIMES MONOCYTES/MACROPHAGES FOR APOPTOTIC CELL DEATH

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The immunomodulatory and antiviral cytokine IFN-β is the first drug to show therapeutic effect in multiple sclerosis (MS), but its mechanism of action remains as enigmatic as the etiology of MS itself. IFN-β has been shown to augment activation-induced T-cell death *in vitro* in MS patients, strengthening the hypothesis that increased T-cell apoptosis should be beneficial in MS. However, in later stages of tissue pathology, T cells are greatly outnumbered by macrophages, which execute the final step in demyelination, i.e. myelin phagocytosis. Therefore, we investigated the effect of IFN-β *in vivo* and *in vitro* upon monocyte/macrophage apoptosis in MS patients. We found that treatment with IFN-β *in vivo* had no effect on annexin V-binding or Fas surface expression in freshly isolated monocytes. However, when comparing monocytes after 40 h of *in vitro* culture, i.e. after differentiation into macrophages, we observed a time-dependent increase in apoptotic cells induced by IFN-beta treatment *in vivo*, from 16.7 (± 4.7)% before treatment to 57.2 (± 18.4)% after three months of treatment. Interestingly, stimulation of the cells with IFN-beta *in vitro* resulted in an even further increase of annexin V binding. Fas expression was strongly increased by treatment *in vitro* but not *in vivo*, which might suggest that the *in vivo* priming effect of IFN-β on monocyte apoptosis occurs independent of Fas upregulation. Thus, our findings extend those of Kaser et al. (1999) to cells of the monocytic lineage and to the *in vivo* level, i.e. monocytes primed by IFN-β treatment undergo apoptotic cell death upon subsequent activation and differentiation. Therefore, triggering of programmed cell death in effector cells *in situ* might be one possible mechanism through which IFN-β exerts its beneficial effect in MS.

00004

MULTIPLE SCLEROSIS: PRO- AND ANTI-INFLAMMATORY CYTOKINES AND METALLOPROTEINASES ARE AFFECTED DIFFERENTIALLY BY TREATMENT WITH IFN-β

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Interferon-beta (IFN-β) has a beneficial influence on the course of multiple sclerosis (MS) and has become standard treatment of this disease, though its mechanisms of action are incompletely understood. This study examines the effect of IFN-β treatment on the cytokines (IL-6, TNF-α, IFN-γ and IL-10) and the metalloproteinases (MMP-3, -7, -9, TIMP-1). IFN-β treatment results in a decrease in numbers of mononuclear cells (MNC) secreting IL-6 and TNF-α and expressing mRNA of MMP-3 and MMP-9 compared to pretreatment levels. On the contrary numbers of MNC secreting IL-10 and expressing mRNA of TIMP-1 were augmented. Effects were similar in patients followed during relapsing-remitting and secondary progressive phase of MS, and for both IFN-β1a and IFN-β1b. Whether the down-regulatory effects on pro-inflammatory and upregulatory effects on anti-inflammatory molecules are a direct result of IFN-β on the immune system or secondary to clinical stabilization of MS pathology induced by IFN-β remains to be evaluated.

00003

EFFECTS OF IFN-β ON MCP-1 EXPRESSION AND PRODUCTION IN RELAPSING-REMITTING MS (RR-MS)

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We have shown that IFN-β treatment decreases RANTES expression and production in peripheral blood adherent monocytes (PBAM) from RR-MS. Aim of this study was to assess in RR-MS IFNβ-1b effects on MCP-1 We established PBAM cultures from 21 RR-MS (9 in relapse, 12 in remission) and 11 RR-MS treated with IFNβ-1b (5 in relapse, 6 in remission). Ten healthy adult subjects served as controls (HC). Antigenic MCP-1 was measured through ELISA (sensitivity: 10 pg/ml) and MCP-1 mRNA detected using semi-quantitative RT-PCR in unstimulated and PHA-stimulated PBAM. Analysis of variance was used to detect differences among disease and treatment status. PHA induced an increase in chemokine production in all groups of patients. MCP-1 production was higher in RR-MS, compared to HC. MCP-1 levels in untreated patients were higher in relapse than in remission. In the stable phase MCP-1 production was higher in IFN β-1b treated than in untreated pts. In relapse MCP-1 production in IFN β-1b treated pts was lower than in all other MS groups. In PHA^+ cultures MCP-1 mRNA expression was higher in RR-MS patients irrespective of disease phase or treatment than in HC. MCP-1 expression was higher in relapse than in remission and in treated than in untreated pts. Our results in untreated pts suggest that MCP-1 plays a role in MS pathogenesis. The effects of IFN β-1b treatment on MCP-1 and RANTES in PBAM cultures from RR-MS are different both during relapses and remissions. Further studies are warranted to clarify chemokine involvement in MS.

00002

CELL RESPONSE TO BETAFERON AS AN INDICATION ON PROGNOSIS IN MULTIPLE SCLEROSIS.

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It is unequivocally evident that finding of methods for individualization of immune therapy in multiple sclerosis (MS) would be important in optimization of the disease treatment. Such an approach was the last years elaborated. It was based on testing in vitro response of cells obtained in whole blood probes to the action of preparations included in a spectrum of clinical use. There were, first of all, interferons (IFNs) and their inducers.

Using this experimental approach we have studied dynamics of IFN system state parameters and a response of cells from the whole blood probes to the in vitro betaferon action in MS patients which received betaferon trial. It has been shown that clinical effect of the preparation was registered when the cells of patients kept in vitro sensitivity to betaferon. Later, in parallel with a positive neurological state dynamics and with normalization of initially decreased IFN system state parameters, a response of cells to the in vitro betaferon action gradually reduced. On the contrary, increase in cell response to betaferon action was registered before the following exacerbation. The fact forced to newly apply the preparation, again with positive clinical effect.

The data obtained may indicate on possibility to use the in vitro cell response to betaferon not only in sorting the responsible patients but also in a prognosis of following clinical improvements or exacerbations.

00001

IFN-β inhibits TNF-α and IL-1β while enhancing IL-1 receptor antagonist production in cell contact-mediated T lymphocyte-signaling of monocytes

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Mainly produced by monocyte-macrophages TNF-α and IL-1β play critical roles in immuno-inflammatory diseases, while the mechanisms which induce cytokine production in monocyte-macrophages in the absence of infectious agents remain elusive. Our previous studies demonstrated that direct cellular contact with stimulated T lymphocytes triggers the production of large amounts of IL-1β and TNF-α in monocyte-macrophages. The present study shows that IFN-β inhibits contact-mediated activation of monocytes by stimulated T lymphocytes whether in co-cultures of living cells or in monocytes activated by membranes of stimulated T cells. In co-cultures of autologous T lymphocytes and monocytes stimulated by PHA, IFN-β inhibited the production of TNF-α and IL-1β by 88 and 98%, respectively, whereas the simultaneous production of IL-1 receptor antagonist (IL-1Ra) was enhanced 2-fold. The effects of IFN-β on the production of TNF-α, IL-1β and IL-1Ra were independent of modulations in IFN-γ, IL-4 and IL-10 production by either T lymphocytes or monocytes. When monocytes were activated by plasma membranes of stimulated T cells, IFN-β slightly inhibited the production of TNF-α and IL-1β, while enhancing 1.5-fold that of IL-1Ra. The latter effect correlated with the persistence of high steady-state levels of IL-1Ra mRNA after 24 h of activation. Membranes isolated from T lymphocytes that had been stimulated in the presence of IFN-β displayed a 80% decrease in their capacity to induce the production of IL-1β and TNF-α in monocytes, whereas IL-1Ra induction was decreased by only 32%. These results demonstrate that IFN-β modulates contact-mediated activation of monocytes by acting on both T lymphocytes and monocytes, decreasing the ability of T lymphocytes to induce TNF-α and IL-1β production in monocytes and directly enhancing the production of IL-1Ra in the latter cells. The fact that these *in vitro* effects of IFN-β corroborate *in vivo* observations in patients treated with IFN-β suggests (i) that inhibition of TNF-α and IL-1β and the stimulation of IL-1Ra production upon contact-mediated T lymphocyte-signaling of monocytes may be relevant to therapeutic efficacy in immuno-inflammatory diseases, and (ii) that the latter mechanism may play an important part in chronic inflammation.

Chemokines

19006

Chemokines

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While the events of leukocyte trafficking may appear intuitive, it has taken over 150 years of research to elucidate the molecular and cellular steps involved in the process of leukocyte migration. The maintenance of leukocyte recruitment during inflammation requires intercellular communication between infiltrating leukocytes and the endothelium, resident stromal and parenchyma cells. These events are mediated via the generation of early response cytokines, e.g., IL-1 and TNF, the expression of cell-surface adhesion molecules, and the production of chemotactic molecules, such as chemokines. The human CXC, CC, C, and CX_3C chemokine families of chemotactic cytokines are four closely related polypeptide families that behave, in general, as potent chemotactic factors for neutrophils, eosinophils, basophils, monocytes, mast cells, dendritic cells, NK cells, T and B lymphocytes. These cytokines in their monomeric form range from 7-10 kD and are characteristically basic heparin-binding proteins. The chemokines display highly conserved cysteine amino acid residues: the CXC chemokine family has the first two NH_2-terminal cysteines separated by one non-conserved amino acid residue, the CXC cysteine motif; the CC chemokine family has the first two NH_2-terminal cysteines in juxtaposition, the CC cysteine motif; the C chemokine has one lone NH_2-terminal cysteine amino acid, the C cysteine motif; and the CX_3C chemokine has the first two NH_2-terminal cysteines separated by three non-conserved amino acid residues. The CX_3C chemokine, Fractalkine, is perhaps the most unique chemokine. Initially described on human non-hemopoietic cells, it can exist as two forms, either as membrane-anchored form or as a shed glycoprotein. The two forms allow this chemokine to function as either a β_1- or β_2-independent adhesion molecule or as a chemoattractant, respectively, for T cells and monocytes. There is approximately 20% to 40% homology between the members of the four chemokine families. This presentation will provide an overview of chemokines and their function.

19007

THE CHEMOKINE-PROTEASE CONNECTION: PROCESSING OF CHEMOKINES BY PROTEASES DIFFERENTLY AFFECTS THEIR INFLAMMATORY AND ANTI-HIV-1 PROPERTIES

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Chemotactic cytokines or chemokines are classified according to the positioning of their conserved cysteines (C, CC, CXC and CX_3C). These mediators exert activities through 7-transmembrane receptors (CCR, CXCR), some of which are co-receptors for HIV-1. Chemokines play an important role in leukocyte migration during inflammation and in physiological leukocyte trafficking, as well as in hematopoiesis and angiogenesis. In contrast to cytokines, which are not affected by the dipeptidyl-peptidase IV/CD26, a minimal cleavage of two NH_2-terminal residues from chemokines by this ecto-enzyme resulted in a drastic reduction in the chemotactic responses to SDF-1 (CXCR4), RANTES (CCR1,3), eotaxin (CCR3) and MDC (CCR4). This negative feedback on the inflammatory response is reflected by a reduction in binding or signaling capacity through the corresponding chemokine receptors. However, truncation of RANTES and MIP-1α/LD78β by CD26 caused an enhanced CCR5 binding efficiency resulting in increased anti-HIV-1 activity. Furthermore, after such processing LD78β showed enhanced chemotactic potency due to more efficient CCR1 binding. This dual effect of proteases as regulators of chemokine activity was extended by the metalloproteinase gelatinase B, which processed IL-8 NH_2-terminally into a more potent neutrophil chemoattractant, whereas other CXCR2 ligands (CTAP-III and GRO) were degraded. A number of chemokines remained unaffected by either gelatinase B or CD26. Finally, for other chemokines, e.g. MCP-1, MCP-2, GCP-2, ENA-78, the underlying mechanism for the observed natural processing is still unknown. It can be concluded that the NH_2-terminal region of chemokines is important for their activity and that the generation of proteolytically processed chemokines serves as a natural system to enhance or decrease inflammation and/or infection by HIV-1.

19004

CHEMOKINE RECEPTORS INTERACT WITH PP2A IN A PHOSPHORYLATION-INDEPENDENT BUT INTERNALIZATION-DEPENDENT MANNER

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The CXC chemokine receptor, CXCR2, is a G protein-coupled seven transmembrane receptor that is expressed in many cell types. Receptor phosphorylation and dephosphorylation have been postulated to play an important role in the regulation of the receptor signaling and trafficking. In this study we demonstrate that CXCR2 interacts specifically with the structural subunit of protein phosphatase 2A (PP2A/A) using the yeast two hybrid screen, GST pull-down, and cell immunoprecipitation assays. The binding of PP2A/A with CXCR2 occurs in the carboxyl terminus of the receptor. CXCR2 co-immunoprecipitates with the core enzyme of PP2A including PP2A/A and the catalytic subunit of PP2A (PP2A/C) in HEK293 cells, in an agonist and time-dependent manner, with maximal binding after 10 min of ligand treatment. Truncation of the C terminal phosphorylation sites, which results in a loss of the agonist-induced phosphorylation, does not affect the receptor binding to the PP2A core enzyme. However, overexpression of dominant negative dynamin 1 (K44A) or impairment of the receptor internalization by mutation of the dileucine motifs in the C-terminus of CXCR2, blocks the receptor binding to PP2A. Immunofluorescence microscopy shows that CXCR2 colocalizes with PP2A in the endosome after agonist exposure. Another chemokine receptor, CXCR4 also interacts with PP2A in a time-dependent manner in response to agonist treatment Impairment of the receptor phosphorylation and internalization by truncation of the C terminal tail of the receptor blocks the receptor binding to PP2A. Overexpression of dynamin 1 (K44A) inhibits this interaction as well. For both receptors, blocking PP2A activity using okadaic acid specifically prevents receptor dephosphorylation and subsequent degradation These data indicate that PP2A is involved in the dephosphorylation of chemokine receptors, and that both CXCR2 and CXCR4 interact with PP2A in a phosphorylation-independent but internalization-dependent manner.

19001

Cooperativity between NF-κB and IRF Factors in RANTES Chemokine Gene Expression Analyzed by *In vivo* Genomic Footprinting

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Virus infection of host cells activates a set of cellular genes including cytokines, interferons and chemokines, involved in antiviral defense and immune activation. Previous studies demonstrated that virus-induced transcriptional activation of a member of the human CC-chemokine RANTES required activation of the latent transcription factors IRF-3 and NF-κB/IκB via post-translational phosphorylation. In the present study, we further characterized the regulatory control of RANTES transcription during virus infection using *in vivo* genomic footprinting analyses. IRF-3, the related IRF-7 and NF-κB are identified as important *in vivo* binding factors required for the cooperative induction of RANTES transcription after virus infection. Using fibroblastic or myeloid cells, we demonstrate that the kinetics and strength of RANTES virus-induced transcription is highly dependent on the pre-existence of IRFs and NF-κB. Use of dominant negative mutants of either IκBα or IRF-3 demonstrated that disruption of either pathway dramatically abolishes the ability of the other to bind and activate RANTES expression. Furthermore, co-expression of IRF-3, IRF-7 and p65/p50 leads to synergistic activation of RANTES promoter transcription revealing a model of virus-mediated RANTES promoter activation which involves cooperative synergism between IRF-3/IRF-7 and NF-κB factors. To further characterize the involvement of NF-κB signaling in RANTES induction, retroviral-mediated transfer was used to introduce a dominant form of IKKγ/NEMO subunit (ΔC-IKKγ) into monocytic U937 cells. ΔC-IKKγ expression inhibits LPS-, TNFα- and virus-induced IKK activity and NF-κB binding activity as well as decreased RANTES gene expression. These experiments suggest a strategy to decrease cytokine production during chronic inflammatory conditions by interfering with IKK signaling.

19005

REGULATION OF CCR6 CHEMOKINE RECEPTOR EXPRESSION AND RESPONSIVENESS TO MACROPHAGE INFLAMMATORY PROTEIN (MIP)-3α/CCL20 IN HUMAN B CELLS

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We analysed the regulation of CCR6 chemokine receptor expression during B cell ontogeny and Ag-driven B cell differentiation. None of the $CD34^+Lin^-$ hematopoietic stem cell progenitors, the $CD34^+CD19^+$ (pro-B) nor the $CD19^+CD10^+$ (pre-B/immature B cells) B cell progenitors expressed CCR6. CCR6 is acquired when CD10 is lost and B cell progeny matures, entering into the $sIgD^+$ mature recirculating B cell pool. CCR6 is expressed by all bone marrow-, umbilical cord blood- and peripheral blood-derived naive and/or memory B cells but is absent from germinal center (GC) B cells of secondary lymphoid organs. CCR6 is downregulated after B cell antigen receptor(BCR)-triggering and remains absent during differentiation into Ig-secreting plasma cells whereas it is reacquired at the stage of post-GC memory B cells. Thus, within the B cell compartment, CCR6 expression is restricted to functionally mature cells capable of responding to antigen challenge. In transmigration chemotactic assays (Transwell system) Macrophage Inflammatory Protein (MIP)-3α/CC Chemokine Ligand 20 (CCL20) induced vigourous migration of B cells with differential chemotactic preference toward $sIgD^-CD44^{high}$ memory B cells. These data suggest that a restricted pattern of CCR6 expression and MIP-3α/CCL20 responsiveness is an integral part of the process of B-lineage maturation and Ag-driven B cell differentiation.

19002

Differential control of chemokine expression by IL-6 and its soluble receptor: A mechanism for regulating leukocyte recruitment during inflammation.

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Peritoneal inflammation is a recurrent complication in end-stage renal failure patients undergoing continuous ambulatory peritoneal dialysis. A diagnostic feature of this condition is an initial intraperitoneal influx of neutrophils that are subsequently replaced by a more sustained population of mononuclear leukocytes. Here we report that interleukin (IL)-6 in combination with its soluble IL-6 receptor (sIL-6R) coordinates this switch in the pattern of leukocyte recruitment. Examination of human peritoneal mesothelial cells (HPMC) showed that IL-6 in the presence of sIL-6R promoted secretion of the mononuclear cell chemoattractant MCP-1, but not RANTES or the neutrophil-activating CXC-chemokines IL-8 and GROα. Interestingly, IL-1β- or TNFα-induced expression of IL-8 and GROα by HPMC was significantly inhibited (50-60%) by the [sIL-6R/IL-6] complex. in contrast, IL-1β-induced expression of RANTES remained unaffected by the active complex. Suppression of neutrophil chemotaxis through sIL-6R signaling was confirmed using a HPMC transwell migration assay. These data suggest that sIL-6R-signalling suppresses neutrophil infiltration, while promoting mononuclear cell recruitment. To explore this possibility, a peritoneal inflammation model based upon the intraperitoneal administration of a *Staphylococcus epidermidis* cell-free supernatant (SES) was established in IL-6-deficient ($IL\text{-}6^{-/-}$) mice. Induction of inflammation by a defined-dose of SES resulted in a similar pattern of leukocyte recruitment as that seen in the human condition. Reconstitution of IL-6 signaling within $IL\text{-}6^{-/-}$ mice was achieved by administering a chimeric sIL-6R-IL-6 fusion protein (Hyper-IL-6: H-IL-6). Simultaneous intraperitoneal injection of SES and H-IL-6 (40 ng/mouse) significantly inhibited neutrophil migration into the peritoneal cavity and was accompanied by a decrease in expression of MIP-2 and KC, the murine homologues of IL-8 and GROα respectively. Studies are currently examining the role of sIL-6R in mononuclear cell infiltration of the peritoneal cavity. Taken together, these data support a pivotal role for the [sIL-6R/IL-6] complex in controlling the pattern of leukocyte recruitment associated with peritoneal inflammation and ultimately sIL-6R may contribute to the resolution of this inflammatory condition. (Supported by a Wellcome Trust Project Grant- SAJ / NT).

19018

TITLE: SERP-1, A UNIQUE VIRAL ANTI-ATHEROGENIC PROTEIN REGULATES PAI-1 EXPRESSION IN HUMAN ENDOTHELIAL CELLS

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Purpose: The plasminogen activators (PA) and the urokinase-type PA receptor (uPAR) complex have chemotactic, adhesive and proteolytic activity. SERP-1 (S-1), a viral PA inhibitor, blocks plaque growth in a rat (R) angioplasty injury model. We have examined the effects of S-1 on rat arteries and cell cultures of human endothelial cells (HUVEC), platelets (P), and monocytes (MC) and rat smooth muscle cells (SMC). Methods: Cellular activation was assessed by membrane fluidity assay. Rat arteries were assessed after angioplasty for tPA, PAI-1 and uPAR by enzyme activity assay, Western blot, Northern blot and RT- PCR (RT-PCR) analysis at 0-24hrs and 10days (120R). Cell cultures were assessed by RT-PCR. Results: S-1 reduced plaque growth after balloon injury (6R) on comparison with controls (6R)($P<0.0001$). S-1 reduced cellular activation responses as determined by membrane fluidity. MRNA was increased for uPAR and PAI-1, and reduced for tPA ($P<0.001$) after S-1 infusion. S-1 increased PAI-1 and reduced tPA protein (32R) and enzyme activity (20R). S-1 also increased PAI-1 mRNA expression in HUVEC cells, but not in SMC or MC. Anti-uPAR and anti-vitronectin antibodies blocked S-1 induced PAI-1 expression in HUVEC cells.
Conclusion: 1. S-1 inhibition of plaque growth is associated with altered cellular activation and upregulation of PAI-1 expression. 2. Antibodies to uPAR and vitronectin block S-1 induced increases in PAI-1 expression.

19009

Production of stromal cell derived factor-1 by mesothelial cells and targeting of B1 lymphocytes population to the peritoneal cavity

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B1 lymphocytes accumulate and proliferate in the peritoneal cavity. Stromal cell-derived factor 1 (SDF-1) is a chemotactic and growth promoting factor for B-cell precursors. It is required for fetal liver B-cell lymphopoiesis, which generates mostly B1 lymphocytes. Using immunohistochemistry with an anti-SDF-1 mAb, we found that SDF-1 was produced by peritoneal mesothelial cells in adult mice. Peritoneal B1 lymphocytes express a functional SDF-1 receptor, as shown by actin polymerization experiments. SDF-1 stimulates *in vitro* the migration, proliferation and survival of peritoneal B1 lymphocytes. Analysis of single cells indicated that only few B1 cells proliferated in the presence of SDF-1, but that these cells underwent several cycles of division. *In vivo* neutralization of SDF-1 for three weeks significantly decreased the number of peritoneal B1 cells. SDF-1 also acts on peritoneal B2 cells. These findings show that after the cessation of B-cell lymphopoiesis in the liver, around birth, the persistence and renewal of B1 cells remains SDF-1-dependent, and targeted to the peritoneal cavity due to mesothelial cell SDF-1 production. Thus, the role of mesothelial cells for B1 cells in adults may be similar to that of SDF-1-producing biliary ductal plate cells in the fetus, and to that of bone marrow stromal cells for B2 cell precursors in adults.

19010

REQUIREMENT FOR CDC42/WASP INTERACTION IN SDF-1-INDUCED LYMPHOCYTE CHEMOTAXIS

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The Wiskott-Aldrich syndrome protein (WASP) together with the Rho family GTPase Cdc42 control stimulus-induced actin cytoskeleton rearrangements that are involved in cell motility.
We report here that lymphocytes from patients suffering from Wiskott-Aldrich syndrome (WAS) display abnormal chemotaxis in response to the T-cell chemoattractant stromal cell-derived factor (SDF)-1. WASP is an effector of Cdc42, and we provide here direct evidence that SDF-1 activates Cdc42 and its downstream effector kinases of the PAK family. We then investigated the specific role of the interaction between Cdc42 and WASP in SDF-1-responsive cells. This was achieved by abrogating this interaction with a recombinant polypeptide (TAT-CRIB), comprising the Cdc42/Rac-interactive-binding (CRIB) domain of WASP and an HIV-TAT peptide that renders the fusion protein cell-permeant. This TAT-CRIB protein was shown to block the chemotactic response of a T cell line to SDF-1. Altogether, these data demonstrate that the interaction between activated Cdc42 and WASP is critical for SDF-1-induced chemotaxis of T cells.

19011

SIGNAL TRANSDUCTION PATHWAYS INVOLVED IN SOLUBLE FRACTALKINE-INDUCED MONOCYTIC CELLS ADHESION.

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Fractalkine displays features that distinguishes it from the other chemokines. In particular, besides to its chemoattractant action it has been shown to promote, under physiological flow, the rapid capture and the firm-adhesion of a subset of leukocytes or intervenes in the neuron/microglia interaction. We verified that indeed the human monocytic MonoMac6 cell line adheres to fibronectin-coated filters in response to soluble fractalkine (s-FKN). We found that s-FKN stimulates, with distinct time-courses, extracellular signal-related kinases (ERK1 and ERK2) and stress-activated protein kinases (SAPK1/JNK1 and SAPK2/p38). Both p60 Src and p72 Syk were activated under s-FKN stimulation with a rapid kinetic profile compatible with a downstream regulation on the MAPK congeners. The use of specific tyrosine kinase inhibitors revealed that the ERK pathway is strictly controlled by Syk, while c-Src up-regulated the downstream SAPK2/p38. In contrast, SAPK1/JNK1 pathway was not regulated by any of these non-receptor tyrosine kinases.
The s-FKN-mediated increased adherence of MonoMac6 cells was partially inhibited by SB202190, a broad SAPKs inhibitor, PD98059, a MEK inhibitor, LY294002, a PI(3)K inhibitor, and a pertussis toxin sensitive G protein. These data highlight that the integration of a complex array of signal transduction pathways is necessary to complete the full s-FNK-dependent adherence of human monocytic cells to fibronectin.

19012

HUMAN IL-8 GENE EXPRESSION INDUCED BY CELL STRESS CONDITIONS IN DIFFERENT CELL LINES.

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Tissue injury is often associated with local neutrophil (PMN) accumulation. Interleukin-8 (IL-8) plays a central role in chemotaxis and activation of PMNs. Recent data showed that cell stress conditions including oxidative stress may induce IL-8 synthesis. We studied the expression of IL-8 in various cell cultures in the presence of substances causing cell stress, including hydrogen peroxide, glucose oxidase, NO-generating compounds, stimuli causing cytoskeletal reorganization (colchicine, vinblastine, cytochalasin B), TNF-α and cationic α-defensin from human PMNs. Human neuroblastoma SK-N-SH expressed IL-8 in the presence of desferoxamin and $CoCl_2$, which mimic the hypoxia, hydrogen peroxide, colchicine, vinblastine and TNF, while NO generating agents inhibited IL-8 expression. Similarly, human myelomonocytic cell line THP-1 enhanced the expression of IL-8 mRNA and protein in the presence of H_2O_2, glucose oxidase, colchicine, vinblastine and cytochalasin B, while TNF had no effect on IL-8 expression in THP-1 cells. In contrast, human umbilical vein endothelium cells showed weak response to chemical compounds mentioned above, while TNF caused high induction of IL-8. It was demonstrated that defensins at subtoxic concentrations 10^{-5}M - $3x10^{-5}$M induced IL-8 production in THP and endothelial cells. Genistein inhibited IL-8 synthesis induced by defensins. These results suggest that cell stress and cell injury may induce IL-8 expression in various cells, but responsiveness to particular stimuli depends on the cell type.

19013

HIV-1 GP120 STIMULATES THE PRODUCTION OF β-CHEMOKINES IN HUMAN PERIPHERAL BLOOD MONOCYTES THROUGH A CD4-INDEPENDENT MECHANISM

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The present study was designed to evaluate the effect of the HIV-1 gp120 envelope glycoprotein on the expression of β-chemokines in cultured monocytes/macrophages. Treatment of either freshly isolated 1-day monocytes or 7-day monocyte-derived macrophages (MDM) with recombinant HIV-1 gp120 protein resulted in a specific and dose-dependent enhancement of secretion of MCP-1, MIP-1β and RANTES as well as in a clear-cut increase in the accumulation of the corresponding transcripts. The expression of these mRNA was increased, but not superinduced, in the presence of cycloheximide. β-chemokine secretion was also induced after exposure of monocyte cultures to AT-2 inactivated R5 and X4 HIV-1 strains, retaining conformational and functional integrity of envelope proteins. The gp120-mediated effect was independent of its interaction with CD4, as preincubation with soluble CD4 did not abrogate β-chemokine induction. Moreover, triggering of CD4 receptor by a specific antibody did not result in any β-chemokine secretion. Interestingly, engagement of CCR5 and CXCR4 receptors by specific antibodies as well as treatment with CCR5 and CXCR4 ligands induced β-chemokine secretion. On the whole, these results indicate that HIV-1 stimulates monocytes/macrophages to produce β-chemokines by a specific interaction of gp120 with HIV-1 co-receptors on the cell membrane. The expression of these related polypeptides may represent an important cellular response for regulating both the extent of viral infection and the recruitment of immune cells.

19014

TNF-α DOWN-REGULATES CXCR4 EXPRESSION IN PRIMARY MURINE ASTROCYTES

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CXC chemokine receptor 4 (CXCR4) is a co-receptor for human immunodeficiency virus (HIV) infection and is believed to be involved in the pathogenesis of AIDS-associated neurologic disorders and brain tumors. The physiological roles of CXCR4 in developmental patterning of the nervous and hematopoietic system; gastrointestinal angiogenesis; and cardiac organogenesis were established by studies in gene-targeted mice. Studies on CXCR4 expression and regulation in neuroepithelial cells are fundamental for understanding its physiopathologic roles in the central nervous system (CNS). By using RNase protection assay and immunohistochemistry analysis, we show that CXCR4 expression by primary mouse astrocytes is suppressed by exposure to tumor necrosis factor-α (TNF-α). TNF-α caused a pronounced down-regulation of CXCR4 mRNA in a dose- and time-dependent manner. TNF-α-mediated decrease of CXCR4 mRNA accumulation resulted in decreased CXCR4 protein expression. As a result, the ability of stromal-derived cell factor-1α (SDF-1α) to induce activation of MAP kinases, Erk1/2 was impaired. The half life of CXCR4 mRNA in the presence and absence of TNF-α stimulation was comparable, suggesting that TNF-α-down-regulated CXCR4 mRNA at the transcriptional level. These results suggest that TNF-α could modulate HIV and brain tumor pathogenesis and immune-mediated inflammation in the central nervous system (CNS) by regulation of CXCR4 expression.

19015

REGULATION OF CHEMOKINE RECEPTOR EXPRESSION ON DIFFERENTIATING DENDRITIC CELL PRECURSORS

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The aim of this study was to analyze the kinetics and developmental regulation of chemokine receptor expression on precursors of myeloid dendritic cells. For this purpose, CD34+ cells were isolated from human cord blood and expanded by culture in the presence of SCF and Flt3-Ligand. Proliferating cells were analyzed by FACS for lineage marker and chemokine receptor expression on day 2, 7, 9 or 12 of culture. At early time points, the cells were mostly negative for CCR5 and CCR6, weakly positive for CCR2 and CXCR3, and the majority stained positive for CXCR4. Beyond day 7 of culture, SCF + Flt3L propagated cells became positive for CCR5 and CCR6, decreased the expression of CCR2 and CXCR3 but maintained a relatively high level of CXCR4 expression. At day 7, 9 or 12, differentiation of proliferating cells was induced by adding GM-CSF + IL-4 (GM4), GM-CSF + TNFα + TGFβ (GTT) of IL-3 + Flt3L (3FL) and the cells were analyzed five to six days later (days 12, 14, or 18). 3FL-supplemented medium supported the differentiation of cells expressing CCR6, that also stained positively for CCR5 and partially for CXCR3 and CCR2. However, these cells – while expressing MHC class II, CD33 and CD123 – mostly lacked DC differentiation markers such as CD1a, CD40, CD80 or CD86. GM4 supported the differentiation of DC marker positive cells with high MHC class II and CCR6 expression, that variably expressed CXCR3 and CCR5, but lacked CCR2. GTT cultured cells developed into immature DC that expressed CXCR4 and CCR6 only. These data showed that expression of CCR6 is a feature of all myeloid progeny of CD34+ cells and does not require particular cytokines such as GM-CSF, TNFα or TGFβ, as was indicated in reports published previously.

19026

EXPRESSION PATTERNS AND BIOACTIVITY OF MURINE SCYB11 (muSCYB11)

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We recently cloned the murine homologue of human SCYB11 (alias ßR1, H174, SCYB9B, I-TAC, IP-9, CXCL11) from IFN-γ/LPS-treated murine RAW264.7 macrophages. Among IFNs, the potential to induce muSCYB11 mRNA levels in RAW264.7 cells was IFN-γ > IFN-ß > IFN-α. This was also observed for induction of muMIG and muIP-10 (Crg-2). IL-1ß and TNF-α had no effect on expression of the three chemokines, whereas LPS from *E. coli* potentiated the effect of IFN-γ. As a single stimulus, LPS induced muSCYB11 and muIP-10 in RAW264.7, J774.1, and dendritic cells (Balb/c) but not in dermal fibroblasts or 3T3 cells. Whereas expression levels of muSCYB11, muMIG and muIP-10 are comparable in cell lines, expression levels of muMIG and muIP-10 extend those of muSCYB11 in various organs from Balb/c mice by several orders of magnitude. Recombinant expression of muSCYB11 in *Drosophila* S2 cells confirmed that the mature protein has a molecular mass of 9103,02 Da, as compared to 9113 Da deduced from the cDNA sequence and 9109,05 Da for synthetic muSCYB11. Calcium mobilization experiments using CHO cells transfected with muCXCR3 showed a maximal response at 30 nM muSCYB11. Cross desensitization studies demonstrated that muSCYB11 stimulated muCXCR3 at much lower doses than muMIG or muIP-10. Chemotactic activity of muSCYB11 was confirmed using murine pre-B 300-19 cells transfected with muCXCR3. When used on cells transfected with human CXCR3, muSCYB11 blocked the chemotactic effect of human SCYB11. In summary, expression patterns and chemotactic activity via CXCR3 of SCYB11, MIG, and IP-10 are highly conserved among rodents and humans, thus opening the possibility to study these chemokines in animal models of human disease.

19017

IDENTIFICATION OF THE N-TERMINAL AMINO ACID RESIDUES ESSENTIAL FOR THE BIOGICAL ACTIVITY OF LEUKOTACTIN-1

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Leukotactin-1 (Lkn-1) binds to both CCR1 and CCR3 and induces chemotaxis and calcium influx in human neutrophils, monocytes, and lymphocytes. Structurally, it belongs to a CC chemokine subfamily, but is distinct from the rest of the CC chemokines in that it has long amino acid residues preceding the first cysteine at the N-terminus, and contains six conserved cysteines. N-terminal amino acids, 1-31, of Lkn-1 were deleted serially and the effects of each deletion were investigated. Intact form of Lkn-1 and its mutants lacking 5, 10, 15, 24, 27, 28, 29, 30 and 31 amino acid residues, respectively, were produced in *Escherichia coli* using T7 polymerase-based expression vector, pET-30Xa/LIC. Soluble recombinant proteins were isolated from the bacterial lysate and assayed for their chemotactic and calcium influx-inducing activities in CCR1 expressing HOS cells. Deletion of N-terminal 28 amino acids did not change the biological activities significantly. Deletion of N-terminal 29 amino acids, however, abolished the biological activities of Lkn-1 completely. Our results indicate that 3 amino acid residues preceding the first cysteine at the N-terminus are essential for the biological activity of Lkn-1.

19019

CXCR4 EXPRESSION IN THE HUMAN OVARIAN PREOVULATORY FOLLICLE.

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T lymphocytes and monocytes recruitment is associated with ovulation, a key step of the ovarian folliculogenesis. Chemokines are well known for their role in recruiting leukocytes and may contribute to the recruitment and local accumulation of leukocytes in human preovulatory follicles. Here we examined the expression of CXCR4 receptors and its ligand, the CXC chemokine stromal cell-derived factor-1alpha (SDF-1) which is presently the only CXCR4 ligand.
Cells were obtained from follicular aspirates collected from *in vitro* fertilization patients, then cultured. mRNA for CXCR4 and SDF-1 were quantified by RT-PCR ; CXCR4 were identified by flow cytometry and we measured by spectrofluometry the intracellular calcium ($Ca^{2+}i$) after adding rhSDF-1.
CXCR4 were detected by flow cytometry on granulosa cells (CD45- cells), T lymphocytes (CD3+ cells) and monocytes/macrophages (CD14+ cells) ; rh SDF-1 induced a rapid increase in $Ca^{2+}i$; after a 72 h culture period CXCR4 disappeared in granulosa cells while they were still strongly expressed in lymphocytes and monocytes. CXCR4 expression in leukocytes was up-regulated by 8 Br cAMP. Transcripts for SDF-1 were detected in cultured cells and increased after a 72 h culture period, suggesting it might be produced by luteinizing granulosa cells.
In conclusion there is probably an ovarian source of SDF-1 at ovulation ; functional CXCR4 are expressed by CD3+ and CD14+ cells and up-regulated by factors released in culture medium from granulosa cells as well as by cAMP. This supports an active role for locally produced SDF-1 in the ovulatory process involving a local accumulation of immune cells.

19025

Transient arrest of rolling leukocytes: a novel way to regulate extravasation?

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Eosinophils, like other leukocytes, follow the multistep model of extravasation to migrate to inflammatory sites. Cells exhibit a rolling interaction with selectin expressing endothelium and need to be activated by inflammatory mediators to firmly adhere to this surface. The chemokine IL-8 is enhanced in pulmonary diseases such as asthma. Therefore, a link between eosinophil influx and IL-8 enhancement has been suggested. Thus far, the effect of IL-8 on unactivated (=unprimed) eosinophils is not known. In this study the effect of IL-8 on eosinophils adhering to physiologic relevant surfaces was investigated. For this purpose eosinophils were incubated in the presence or absence of blocking anti β2- and anti α4- integrin monoclonal antibodies (Mab) or Pertussis Toxin (PT) and perfused over 7h TNF-α-activated HUVEC in an in vitro flow chamber model. Subsequently, rolling eosinophils were exposed to IL-8 (10^{-8}M) or eotaxin (10^{-8}M). We show that IL-8 can induce a transient arrest of unprimed eosinophils which were rolling on TNFα-activated HUVEC in an in vitro flow chamber. Blocking antibodies against both β2- and α4 integrins and also preincubation of the eosinophils with PT inhibited the IL-8 induced transient arrest. These data show that resting eosinophils respond to IL-8 only when the cells adhere to physiological surfaces. The induction of a transient arrest provides a new level of regulation of leukocyte adhesion under flow conditions.

19021

CHEMOKINE AND CHEMOKINE RECEPTOR EXPRESSION ON HODGKIN'S DISEASE TUMOR CELLS

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Hodgkin's disease (HD) is characterized by small numbers of HD tumor cells, the Hodgkin/Reed-Sternberg cells (H/RS), which are embedded in a reactive infiltrate. This is composed of lymphocytes, plasma cells, histiocytes, granulocytes and eosinophils. The infiltrating cells rather support than eradicate the tumor cells. Recent in situ studies have suggested a role of chemokines in the pathogenesis of HD. To further elucidate the role of H/RS tumor cells, we compared the expression of chemokines and their receptors in different H/RS cell lines (L1236, L428, KM-H2 and HDLM-2) with cell lines derived from Burkitt's lymphoma (BL-2, BL-30, BL-41), a B cell derived lymphoma with different histological features. As shown on mRNA (RNAse protection assay) and protein (ELISA) levels, the B-cell derived H/RS cell lines L1236, L428 and KM-H2 expressed significantly higher amounts of the CC-chemokine RANTES and the CXC-chemokine IP-10 than the BL derived cell lines. Similar differences were found for receptors of both the CC- and CXC chemokine families. Our data suggest that H/RS cells constitutively express chemokines and chemokine receptors which both distinguish them from other B cell derived lymphoma types.

19022

IDENTIFICATION OF A COMMON RECEPTOR, RAT CXCR2 FOR THREE TYPES OF CYTOKINE-INDUCED NEUTROPHIL CHEMO-ATTRACTANTS (CINCs)

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Cytokine-induced neutrophil chemoattractant (CINC)-1, CINC-2 (CINC-2α and -2β) and CINC-3/macrophage inflammatory protein-2 (MIP-2), members of the CXC chemokine family, are potent chemotactic factors for rat neutrophils. In order to identify the receptor for CINCs, a rat CXC chemokine receptor 2 (CXCR2) gene was cloned and expressed in HEK293 cells. CINC-1, CINC-2 and CINC-3/MIP-2 induced the transient increase in intracellular free-Ca^{2+} concentration ($[Ca^{2+}]i$) dose-dependently in CXCR2-transfected cells, whereas formyl-methionyl-leucyl-phenylalanine (FMLP) did not. CINC-3 induced enhancement of $[Ca^{2+}]i$ more potently than CINC-1 and CINC-2, and desensitized $[Ca^{2+}]i$ induced by CINC-1 and CINC-2, which were essentially identical to those observed in rat neutrophils. In addition, anti-CXCR2 serum inhibited neutrophil chemotactic activities of three types of CINCs almost completely. The mutant CINC-3, whose N-terminal amino acid sequence (SELR) was replaced to AAR, lost chemotactic activity of its own but inhibited that of CINC-1 and CINC-2 potently, and that of CINC-3 weakly. Similarly, CINC-3 (4 - 69), whose N-terminal 3 amino acid residues (SEL) were deleted, had no chemotactic activity but inhibited $[Ca^{2+}]i$ induced by CINC-1. The results indicate that rat CXCR2 on neutrophils is the unique receptor for all three types of CINCs.

19023

A SENSITIVE ENZYME-LINKED IMMUNOSORBENT ASSAY FOR HUMAN 77 AMINO-ACID ALA-FORM OF HUMAN INTERLEUKIN-8.

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IL-8 plays a central role in neutrophil chemotactic responses. IL-8 exists in two forms: 77 and 72 amino-acid forms (Ala-IL-8_{77} and Ser-IL-8_{72}), which are generated by differential cleavage of common progenitor protein. Ala-IL-8_{77} was reported to be produced predominantly by endothelial cells, while Ser-IL-8_{72} is produced by macrophages. Recent data imply that biological activity of Ala-IL-8_{77} may differ from that of Ser-IL-8_{72}: only Ala-IL-8_{77} induces apoptosis in leukemic cells in vitro and in vivo (Y. Terui et al., Blood, 92: 2672-2680, 1998).

The goal of this study was to develop an immunoassay capable to detect Ala-IL-8_{77}, since existing immunoassays can not distinguish two forms. For this purpose we produced mouse monoclonal antibody specific to 5 N-terminal amino-acids of human Ala-IL-8_{77}. Antibody recognized and neutralized chemotactic activity of synthetic and cell-produced Ala-IL-8_{77}, but did not react to Ser-IL-8_{72}. Sensitive specific to Ala-IL-8_{77} ELISA was developed. Ala-IL-8_{77} was detected in supernatants of endothelial cells, human leukocytes and tumor cell lines. It was shown that accumulation of Ala-IL-8_{77} in culture medium is not a constitutive property of given cell type, but rather depends on culture conditions, while Ala-IL-8_{77} was primarily produced. Various Ala-IL-8_{77} levels were detected in human body fluids in normal state and pathology, thus proving that this form of IL-8 plays a physiological role in vivo.

19024

SIGNAL TRANSDUCTION INVOLVED IN MCP1-MEDIATED MONOCYTIC TRANSENDOTHELIAL MIGRATION.

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Monocyte chemoattractant protein-1 (MCP-1) is a major chemoattractant for monocytes and T lymphocytes. The MonoMac6 cell line was used to examine MCP-1 receptor-mediated signal transduction events in relation with MCP-1-mediated monocytic transendothelial migration. We report here that MCP-1 stimulates, with distinct time-courses, extracellular signal-related kinases and stress-activated protein kinases (JNK1 and p38). JNK1 activation was blocked by piceatannol, indicating it is regulated by Syk kinase while p38 was inhibited by PP2, revealing an upstream regulation by Src-like kinases. In contrast, ERK activation was PP2 and piceatannol insensitive. We found that pertussis toxin abrogated MCP-1-induced ERK activation, but was without any effect on JNK1 and p38 activation. These results underscore the major implication of Go/i proteins and non-receptor tyrosine kinases in the early MCP-1 signaling. In addition, MCP-1-mediated chemotaxis and transendothelial migration were significantly diminished by high concentration of SB202190, a broad SAPKs inhibitor, or by SB203580, a specific inhibitor of p38. PTX treatment of MonoMac6 cells also prevented up to 50% of the migratory response and gave rise to a cumulative effect when added with SB202190, suggesting the requirement of the coordinated action of non-overlapping signal transduction to produce an efficient MCP-1-mediated monocytic migration.

19020

MOLECULAR COMPARISON OF HUMAN AND MURINE SCYB11

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Among CXC chemokines, human SCYB11 (alias ßR1, H174, SCYB9B, I-TAC, IP-9, CXCL11), MIG, and IP-10 constitute an individual branch of the phylogenetic tree. The three genes are clustered on chromosome 4q21.2. We recently identified the murine homologue of SCYB11. Murine and human SCYB11 share 63 % nucleotide and 68 % amino acid sequence identity. In both species, mRNA forms using the classical and additional polyadenylation sites do occur. Comparison of human and murine SCYB11 showed organization in 4 exons with similar exon/intron boundaries for both. Intron 1 of murine SCYB11 carries an additional 201-bp stretch of repetitive sequences with high cryptic simplicity. Also murine SCYB11 shares an individual phylogenetic branch together with murine MIG and murine IP-10 (Crg-2). Furthermore, murine SCYB11 is located on chromosome 5E3, a region orthologous to the position of human SCYB11. Fiber fish experiments showed that the arrangement of murine SCYB11, MIG and IP-10 is identical to that of the respective human genes. In both species, the three genes cluster within a region of about 30 kb. Within the promoter region of murine and human SCYB11, about 150 bp are homologous and putative transcription factor binding sites as well as the TATA box are conserved. Taken together, these results strongly support the hypothesis that SCYB11, MIG and IP-10 result from gene duplication which took place before emergence of muroid rodents.

Genomic structure and function of interferon and cytokine genes

00001

IL-15 GENE EXPRESSION IN RAT PERIPHERAL BLOOD MONONUCLEAR CELLS AND SKELETAL MUSCLE TISSUE: DETECTION OF NOVEL TRANSCRIPTS GENERATED BY ALTERNATIVE SPLICING.

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IL-15 synthesis is regulated by several post-transcriptional mechanisms. In humans, an extra exon (119 bp) encoded in the classic intron 4 introduces a premature stop codon followed by a translation start site, producing a shorter signal peptide and yielding a mature form with intracellular localization. In mice, a shorter signal peptide is introduced by an alternate exon 5 generated by alternative splicing 136 bp upstream of the classic 5' end of exon 5. Alternate IL-15 transcripts have not been described in rats. To gain insights into the post-transcriptional regulation of IL-15, we searched for new IL-15 transcripts in rat soleus muscle and peripheral blood mononuclear cells (PBMC). DNA-free RNA was reverse-transcribed and subjected to PCR. Primers specific for exons 4 and 8 amplified the expected full length mRNA and 2 novel transcripts due to an alternate acceptor splicing site within exon 7. Loss of the 5' end of exon 7 created an in-frame deletion removing 2 of the 4 cysteine residues in mature IL-15. The shorter transcript containing exon 4, alternate exon 7 and exon 8 may encode a peptide unrelated to IL-15 due to a frameshift in alternate exon 7. In PBMC and muscle, the majority of the transcripts contained exons 1 and 3, but not exon 2. However, transcripts starting with exon 2 (absence of exon 1) were detected at higher levels in PBMC than in muscle, suggesting the existence of two putative transcription start sites. In PBMC, LPS induced an accumulation of transcripts starting with exon 2, but did not affect levels of transcripts starting with exon 1. In soleus muscle from rats with adjuvant arthritis, levels of transcripts starting with exon 1 were elevated, but not those of transcripts starting with exon 2. Since rat exon 1 contains five upstream AUGs, the preferential accumulation of IL-15 mRNAs starting with exon 2 in LPS-stimulated PBMC should promote translation whereas the preferential use of exon 1 by soleus muscle in arthritis rats should impair IL-15 synthesis. Despite the difference in 5'UTR, transcripts containing the classic or alternate exon 7 were elevated in LPS-stimulated PBMC and soleus muscle from arthritis rats, when compared to their respective controls. These novel transcripts underline the importance of alternative splicing in the regulation of IL-15 synthesis. Further studies will address the biological significance of the transcript generated by in-frame partial deletion of exon 7.

Cytokines in neurological disease (includes MS and EAE)

00004

LEVELS OF CYTOKINE SECRETING MONOCYTES AND MONONUCLEAR CELLS OVER THE COURSE OF STROKE

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Stroke is a common cause of death and disability in our society. Stroke is associated with changes in immune responses within the central nervous system as well as systemically. In this study, blood monocytes were separated and examined in parallel with corresponding mononuclear cells (MNC) from patients with stroke sampled within 4 days and 1-3 months after onset of symptoms and analysed for numbers of cells secreting TNF-α, IL-12, IL-6 and IL-10 using ELISPOT assays. Numbers of both monocytes and MNC secreting TNF-α, IL-12 and IL-6 were elevated in patients with stroke during the acute phase compared to healthy controls, while no differences were observed between patients with stroke examined during convalescence and controls. IL-10 secreting MNC but not monocytes were elevated during the acute phase of stroke, while levels of IL-10 secreting monocytes did not differ between groups. These data strengthen the notice that systemic immune aberrations occur during the acute phase of stroke. The increase of TNF-α, IL-12 and IL-6 secreting, circulating monocytes indicate the involvement of such cells in the immune responses observed in patients with stroke.

00005

ANTI-IFN-γ HAS A POSITIVE EFFECT IN SECONDARY PROGRESSIVE MULTIPLE SCLEROSIS (A PILOT STUDY OF ANTIBODIES TO IFN-γ AND TNF-α, ALONE AND COMBINED)

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One of us (S Skurkovich, *Nature*, 1974) proposed that a disturbance of interferon (cytokine) synthesis can lead to autoimmune disease and removal of this cytokine can be therapeutic. Postulating that interferon (IFN)-γ synthesis may be a basis for the pathology of multiple sclerosis (MS), we treated 83 patients (60 in a randomized, double-blind, placebo-controlled study) with active secondary progressive MS with a short course of polyclonal antibodies (abs) to IFN-γ, to tumor necrosis factor (TNF)-α and to both cytokines together. Placebo was human albumin. The observation included analysis of functional (FS) and disability (EDSS) scores, accompanied by interval determinations of lymphocyte subpopulations, cytokine production levels, MRI (analyzing number active lesions with enhancement and T2-lesions), and evoked potentials. It was found that 6 and 12 months after the treatment course only patients receiving abs to IFN-γ showed statistically significant improvement of clinical parameters in comparison to the placebo group. At the 12-month point, the time free of progression was significantly longer in the abs to IFN-γ group, as indicated by Kaplan-Meier survival analyses showing significantly less probability for these patients to progress in disability during the observation period. Significant reduction in number of MRI active lesions 6 months after treatment was also seen only in the group receiving abs to IFN-γ. These positive clinical data were supported by changes in cytokine status - a decrease in IL-β, TNF-α, IFN-γ concentration in supernatants of activated blood cells of the MS patients followed by an increase in TGF-β production. Clinical and MRI effects were most prominent 6 months after the course of abs to IFN-γ, while changes in cytokine production - one month later. Long-term administration of humanized mabs to IFN-γ and its receptors is planned.

00006

NEUROCYSTICERCAL ANTIGEN INDUCES SECRETION OF IL-8 & MCP-1 FROM ASTROCYTES VIA A NETWORK INVOLVING MONOCYTE-DERIVED TNF-α.

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Neurocysticercosis (NC), infection of the central nervous system (CNS) by larvae of the pork tapeworm *Taenia solium*, is a major cause of epilepsy worldwide (25% in Peru). Larval degeneration, which is one complication of therapy, results in inflammatory cell influx that can cause clinically dangerous elevation of intracranial pressure. The role of chemokine and cytokine networks in control of cell influx is unknown We investigated gene expression by RNase protection assay and secretion by ELISA of prototypic CC & CXC chemokines, MCP-1 and IL-8 in: (a) human monocytes stimulated with parasite antigen extract (TsAg) and (b) astrocytes (the U373MG cell line) stimulated with conditioned media taken at 8h from TsAg-infected monocytes (CoMTs). Stimulation of monocytes by TsAg caused secretion of IL-8 by 4h reaching maximal by 24h (147 ± 12ng/ml). MCP-1 was initially secreted at 8h, had increased by 24h (78.75 ± 11.95ng/ml) and was maximal after 48h (267 ± 5.66ng/ml). There were corresponding changes in IL-8 and MCP-1 mRNA accumulation. Direct stimulation with TsAg elicited no significant secretion from astrocytes. In contrast, stimulation with CoMTs caused secretion of both MCP-1 (101.33 ± 3.51ng/ml) and IL-8 (181.67 ± 7.37ng/ml) after 24h. Pre-incubation with 1μg/ml anti-TNF-α decreased MCP-1 secretion by 73% and IL-8 secretion by 31%, with no further reduction at 100μg/ml. IL-1RA did not alter chemokine secretion. These data demonstrate that *T.solium* Ag directly drives monocyte and indirectly drives astrocyte IL-8 and MCP-1 secretion. TNF-α, a key regulator of pro-inflammatory cytokines in the CNS, is a pivotal mediator in networks causing such chemokine secretion and may be critical in tissue damage in NC.

00008

SOMATIC GENE THERAPY APPROACH FOR AMELIORATION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS BY TANSFORMING GROWTH FACTOR β1 GENE LOCAL DELIVERY.

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The aim of the present study is to elaborate gene therapy approach to treatment for multiple sclerosis (MS) – an inflammatory putative autoimmune demyelinating disorder of the central nervous system.TGFβ1 is one of the key mediators of the immune response that interfere with demyelination process during MS and its animal model – experimental autoimmune encephalomyelitis (EAE). We investigated the possibility of somatic gene therapy approach to EAE treatment using human TGFβ1 precursor (pTGFβ1) gene.

We have made a retrovirus vector containing the pTGFβ1 gene. The cDNA for pTGFβ1 was cloned into polylinker region of pBabe-neo vector. The expression of the pTGFβ1 gene is from 5'- LTR promoter-enhancer region. The gene for neomycin phosphotransferase was included as a selection marker. The recombinant vector DNA was introduced by calcium-phosphate transfection into ecotropic and amphotropic packaging cell lines PA317, GPE-86 and Fly-MoLV. In case that conditional media from each of three packaging cell lines were used to infect rat embryo fibroblast line REF 52, the titers of replication-deficient vector virus particles were similar (0.9-1.5x10^5 CFU/ml). Transfer and expression of TGF β1 transgene *in vitro* was analysed by the methods of RT-PCR, ELISA and TGF β1 activity - by a bioassay, measured by its antiproliferative effect on the mink lung epithelial cell line Mv 1 Lu.

The pTGFβ1 gene-containing retrovirus particles were used to infect primary fibroblasts and lymphocytes of DA rats. This rat strain is known to be susceptible to the induction of EAE. The preliminary data indicate that intravenous injection of pTGFβ1-transfected syngeneic lymphocytes into immunized animals prior to EAE beginning (1-2 days) results in clinical score reduction comparing to the control untreated animals.

00003

Influence of the antidepressant rolipram on the cytokine network.

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Abstract
We have previously shown that antidepressant drugs have negative immunoregulatory effects. Selective Serotonin Reuptake inhibitors, Tricyclic Antidepressants and Serotonin Noradrenaline Reuptake inhibitors -in physiological concentrations- significantly reduced the secretion of Interferon-γ (IFN-γ) and increased that of Interleukin-10 (IL-10) in stimulated human whole blood. Rolipram is an inhibitor of phosphodiesterase type IV (PDE-IV), but is also known as an antidepressant drug. It displays anti-inflammatory effects, e.g. reduction of disease symptoms in EAE rats and inhibition of IFN-γ and Tumor Necrosis Factor α (TNF-α) production in macrophages.
In the present study, we examined the effects of rolipram on the production of several pro- (IFN-γ, TNF-α, IL-6) and anti-inflammatory (IL-10, IL-1Receptor Antagonist (IL-1RA)) cytokines in stimulated human whole blood. Blood from 20 healthy subjects was diluted 1:4 in medium and stimulated with LPS (5 μg/ml) and PHA (1 μg/ml) in the presence or absence of $2x10^{-7}$, $2x10^{-6}$ and $2x10^{-5}$ M rolipram.
Rolipram significantly and dose dependently inhibited the production of IFN-γ and TNF-α as compared to the positive control. The IFN-γ/IL-10 ratio is significantly reduced in all concentrations as compared to controls. The secretion of IL-1RA was significantly decreased at $2x10^{-6}$ and $2x10^{-5}$ M. The production of IL-6 was significantly reduced at $2x10^{-6}$ M, but enhanced at $2x10^{-5}$ M.
It is concluded that rolipram has negative immunoregulatory effects, since it reduces the IFN-γ/IL-10 ratio and monocytic products, such as IL-1RA and TNF-α. It is hypothesised that rolipram, like other antidepressants, exerts its antidepressant effects by modulating the cytokine network.

00002

CYTOKINES IN REMITTENT STAGE OF MULTIPLE SCLEROSIS

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THE AIM: To estimate the role of disturbances in cytokine network in pathogenesis of MC and to select data, which may predict exacerbation of illness and clinical manifestation.
MATERIALS AND METHODS. 70 patients in remittent stage of multiple sclerosis were observed during last 5 years any times. Males and females were from 17 to 40 years old. IL-1β, IFN-α, IFN-γ, IL-8, IL-6, IL-4, IL-2, TNF-α, ILra and IL-10 in sera samples and supernatants of 18-hours culture of patient's whole blood were measured by ELISA. Production of inflammatory and anti-inflammatory cytokines in vivo and in vitro by blood cells was compared with proliferation, induced by basic myelin protein (BMP) in 72-hours cell culture. Proliferation and apoptosis of PBMC to BMP were investigated by DNA flow cytometry. CD3, CD4, CD8, CD16, CD20, HLAII, CD25, CD95 cells in peripheral blood were investigated by flow cytometry. HLA –A, -B, -DR typing was done.
RESULTS. Th2 type of immune answer in patients with MS was more advantageous than Th1- remissions were longer. HLA-DR2 is the bad prognostic marker for St. Petersburg patients with MS. The prevalence of inflammatory cytokines contrary to anti-inflammatory cytokines was the poor prognostic criterion in MS exacerbation. Augmentation of TNF-α production in vivo and in vitro correlated with significant PBMC proliferation to BMP and appearing of apoptotic peak in DNA flow cytometry. Prolonged effective therapy with IFN-β or IFN-α conduct to decline of inflammatory cytokines production. Augmentation of inflammatory cytokine production (particularly TNF-α), proliferation and appearance of apoptotic high peak in DNA cytometry to BMP predict clinical manifestation of illness in MS patients.

00001

DIFFERENTIAL EFFECT OF IFN-α AND IFN-β ON LYMPHOCYTE ACTIVATION IN ASYMPTOMATIC HTLV-I SEROPOSITIVE INDIVIDUALS AND HAM/TSP PATIENTS.

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Infection with HTLV-1 provokes strong lymphocyte activation, characterized by spontaneous proliferation and increased cytokine secretion (IFN-γ, IL-2, and several other ILs). IFN-α has shown significant clinical benefit in two HTLV-I-associated pathologies, adult T-cell leukemia (ATL) and HTLV-I-associated myelopathy (HAM/TSP), whereas IFN-β is widely used in multiple sclerosis, another neurodegenerative disease. In this study, we have compared the effect of IFN-α and IFN-β *in vitro* on cytokine secretion and lymphocyte proliferation in HAM patients and asymptomatic seropositive individuals. We found that neither IFN-α, nor IFN-β modulated the exacerbated IFN-γ production (>2 ng/ml) observed in HAM patients, or the low and variable IFN-γ production in asymptomatics (<0.5 ng/ml). On the other hand, IFN-α, but not IFN-β, showed a modest inhibitory effect on spontaneous lymphoproliferation in asymptomatic individuals. In HAM patients, IFN-α demonstrated a discrete but significant inhibitory effect (20+/-9 %, $p<0.03$), whereas IFN-β displayed a profound anti-proliferative effect (69+/-4 % of inhibition, $p<0.001$). Inhibition of proliferation correlated with increased apoptosis (measured by annexin V staining) induced by both IFNs. Surprisingly, anti-CD3 stimulation induced an even further decrease in proliferation in most patients tested, correlated to a strong increase in apoptosis. In contrast to published findings in normal donors, IFN-α did not significantly augment anti-CD3-induced lymphocyte apoptosis in these patients.
In conclusion, IFN-α and IFN-β display differential biological activities, at least *in vitro*, in distinct patient groups. Our results suggest that IFN-β might possibly be considered as a complementary or alternative therapy in HAM/TSP patients.

00007

NEURONAL INTERLEUKIN-1β IN THE RAT HYPOTHALAMUS: A ROLE IN PHYSIOLOGY?

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Various immune stimuli as well as brain damage result in an induction of interleukin-1β (IL-1β) in glial cells in the central nervous system which may contribute to brain-mediated responses during illness (eg. fever) and to neuropathology. We demonstrate that small amounts of IL-1β are constitutively expressed in the brain during non-pathological conditions. In the hypothalamus of non-compromised adult rats, IL-1β immunoreactivity is present mainly in oxytocin neurons in the paraventricular and supraoptic nucleus. In addition, IL-1β mRNA is expressed in tissue punches of the paraventricular or supraoptic nucleus. Furthermore, IL-1β positive nerve endings are detected in the posterior pituitary gland, a major projection site of oxytocin neurons. This raises the question whether neuronal IL-1β is transported to the posterior pituitary gland. Hypothalamic lesion studies show accumulation of IL-1β immunoreactive in nerve fibers proximal to the lesion which indicates that neuronal IL-1β is indeed subject to axonal transport. The subcellular distribution of IL-1β in these neurons as studied by confocal laser scan microscopy show that IL-1β is present in or associated with particles which may be of the lysosome type and released by exocytosis. Because functional IL-1 type I receptors are present in the posterior pituitary gland, we hypothesize that IL-1β is released from oxytocin terminals in the posterior pituitary gland and is involved in local actions. This is supported by our pilot observation that suckling reduces oxytocin immunoreactivity in the posterior pituitary gland of the mother which can be inhibited by IL-1ra pretreatment.
We conclude that IL-1β is expressed in hypothalamic neurons under normal physiological conditions. This neuronal IL-1β can be transported to the posterior pituitary gland and may have local actions on hormone release.

14005

REGULATION OF PRO-INFLAMMATORY GENE EXPRESSION BY HUMAN TOLL

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Drosophila Toll mediates dorsal ventral patterning and immune responses. Several human forms of Toll (hToll) have now been cloned and show intriguing similarities to the type 1 IL-1 receptor. IL-1 is a major player in neuropathogenesis. It activates the transcription factor NFkB and induces chemokines and adhesion molecules in resident cells of the brain. This facilitates the cerebral recruitment of leukocytes thus promoting neuropathology. Interestingly hToll activates NFkB and induces IL-8 and co-stimulatory molecules involved in specific immunity. This study investigates the ability of one of the human forms of Toll (hToll4) to mimic IL-1 in its capacity to induce pro-inflammatory genes in astrocytes. 1321N1 astrocytoma were electroporated with a constitutively active form of hToll4 and luciferase reporter genes whose expression were regulated by NFkB, AP-1 and ICAM-1 promoters. hToll4 activated NFkB and ICAM-1 promoters as judged by increased expression of respective reporter constructs. The ICAM-1 promoter was insensitive to hToll4 when the kB site of the latter was mutated. The anti-inflammatory glucocorticoid dexamethasone inhibited the ability of hToll4 to activate the ICAM-1 promoter but was ineffective in regulating IL-1 activation of the same promoter. The inhibitory effects were independent of NFkB but may be mediated by AP-1. This study demonstrates that hToll4 can mimic IL-1 in its ability to activate promoters which regulate pro-inflammatory genes in astrocytes. This promotes hToll4 as another potential trigger of cerebral inflammation and increases our understanding of molecular mechanisms regulating such pathogenic effects.
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Regulation of cytokine and interferon mRNA stability

20003

CONTROL OF CYTOKINE mRNA TURNOVER BY STRESS SIGNALING PATHWAYS.

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A hallmark of inflammatory and immune reactions is the strong and rapid induction of cytokine formation. Accumulating evidence indicates that expression of their genes is controlled at multiple levels, assigning a pivotal role also to post transcriptional mechanisms. Conceivably, a short mRNA half life contributes to the low or undetectable expression levels in unstimulated cells. On the other hand, an increase in mRNA half life upon activation synergizes with its synthesis, resulting in the rapid accumulation of mRNA as a prerequisite for synthesis of high amounts of protein. To study this level of control, IL-6 and IL-8 mRNAs were expressed in HeLa cells using a tetracycline regulatable promoter system. The kinetics of disappearence of the mRNAs upon transcriptional shut-off by tetracycline indicated a high basal degradation rate. IL-1, a potent inducer of IL-6 and IL-8, strongly activates several stress signaling pathways. By expressing constitutively active as well as dominant negative forms of various stress-activated protein kinases IL-1 was found to induce mRNA stabilization through a signaling pathway involving the MAP triple kinase TAK1, p38 MAP kinase and the p38 MAP kinase-activated protein kinase 2 (MK2). Destabilization and signal-induced stabilization was transferred to the stable β-globin mRNA by fragments of IL-8 and IL-6 mRNAs that contain A+U rich regions, and also by defined A+U rich elements (ARE) of the c-*fos* and GM-CSF mRNAs. mRNA stabilization by UV-light, another potent activator of stress-signaling pathways, was insensitive to dominant negative p38 MAP kinase and MK2 and showed a wider transcript selectivity, indicating that it occurs through a different mechanism. Further information on the cis-acting elements and trans-acting factors involved in this type of regulation will be discussed.

20004

MODULATION OF AN AU- RICH ELEMENT BINDING ACTIVITY BY IL-10 IN MOUSE MACROPHAGES.

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IL-10 is widely known to inhibit pro-inflammatory cytokine gene expression in mononuclear phagocytes. This is achieved by inhibiting transcription and/or by selectively destabilizing target mRNAs. IL-10-mediated destabilization of the mouse KC (GRO-α) chemokine mRNA depends upon a cluster of AUUUA sequence elements (AREs) located in the 3' untranslated region (3'UTR) of this mRNA. Cytosolic extracts from untreated RAW264.7 cells can form a specific complex with a radiolabeled RNA fragment containing wild type KC 3'UTR as measured by EMSA; this binding activity is not observed with an RNA in which the ARE cluster has been mutated (mutant KC3'UTR) and is dramatically reduced in extracts from IL-10-treated cells. Furthermore, unlabeled fragments of 3'UTR RNA effectively competed the binding while mutant KC3'UTR was without effect. IL-10 inhibits ARE-specific binding activity in a stimulus-, dose- and time-dependent manner. The effect of IL-10 appears to operate on ARE binding activity in the cytoplasm as similar activity in nuclear extracts is essentially unaltered by IL-10. The RNA-protein complex contained a protein of 36 kDa as determined by UV radiation-induced crosslinking of the specific RNA-protein complex followed by SDS-PAGE. These findings suggest that the ability of IL-10 to destabilize target mRNAs may involve modulation of the activity of a 36 kDa ARE binding protein.

20005

THE MAPKKK TAK1 PLAYS A CENTRAL AND NON-REDUNDANT ROLE IN COUPLING THE IL-1 RECEPTOR TO BOTH, TRANSCRIPTIONAL AND RNA-TARGETTED MECHANISMS OF GENE REGULATION

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Mechanisms of fulminant gene induction during an inflammatory response were investigated using expression of the chemoattractant cytokine interleukin (IL-)8 as a model. Recently we found that coordinate activation of NF-kappaB and c-Jun N-terminal protein kinase (JNK) is required for strong IL-8 transcription, whereas the p38 MAP kinase (MAPK) pathway stabilizes the IL-8 mRNA. It is unclear how these pathways are coupled to the receptor for IL-1, an important physiological inducer of IL-8. Expression of the MAP kinase kinase kinase (MAPKKK) TAK1 together with its coactivator TAB1 in HeLa cells activated all three pathways and strongly induced transcription from a minimal IL-8 promoter as well as stabilization of a reporter mRNA containing IL-8-derived regulatory mRNA sequences. TAB1-TAK1-induced transcription was blocked by a kinase-inactive mutant of JNK2, but not by kinase-inactive p38 MAPK. On the other hand, TAB1-TAK1-induced mRNA stabilization was blocked by a kinase-inactive mutant of p38 MAPK but not by kinase-inactive JNK2. Thus TAK1 is sufficient to activate NF-kappaB + JNK2-mediated transcriptional effects as well as p38 MAPK pathway-mediated mRNA stabilization. Moreover, expression of a kinase-inactive mutant of TAK1 largely blocked IL-1-induced transcription and mRNA stabilization. Truncated TAB1, lacking the TAK1 binding domain, or a TAK1-derived peptide containing a TAK1 autoinhibitory domain were also efficient in inhibition. These data indicate that the previously described three pathway model of IL-8 induction is operative in response to a physiological stimulus, IL-1, and establish TAK1 as the MAPKKK coupling the IL-1 receptor to both, transcriptional and RNA-targetted mechanisms mediated by the three pathways.

20007

INCREASED INTERLEUKIN-10 mRNA STABILITY IN MELANOMA CELLS VERSUS NORMAL MELANOCYTES IS FUNCTIONALLY ASSOCIATED WITH DECREASED LEVELS OF A+U-RICH ELEMENT BINDING FACTORS.

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Immune responses have been classified into type I and type II, regulated by Th1 and Th2 cells, respectively. Some pathological conditions involve an abnormal production of Th1 and Th2 cytokines. One of the cytokines produced by Th2 cells is IL-10, which is an important physiological regulator of inflammatory and immune responses. Elevated levels of IL-10 expression were reported to be associated with a variety of tumors. Since IL-10 is a potent inhibitor of many immune responses, it may contribute to tumor escape from immune surveillance. One mechanism controlling the levels of gene expression is RNA turnover. Rapid mRNA decay is controlled by interactions between A+U-rich elements (ARE) in the 3'-untranslated region (UTR) and RNA-binding proteins. Analysis of IL-10 mRNA identified an ARE in the 3'UTR. The present study was designed to characterize the mechanism of mRNA decay for IL-10, and whether any modification of this mechanism may explain the difference in IL-10 expression in tumors versus normal cells, namely melanoma cells versus melanocytes. We found that the mRNA half-life in a melanoma is twelve-fold longer than in normal melanocytes. We also identified distinct destabilizing determinant(s) in the first 313nt of the IL-10 3'UTR. To determine the specific factor(s) associated with this region that may be responsible for IL-10 mRNA degradation, we have utilized RNA gel shift assays. We identified IL-10 ARE-binding complexes in normal neonatal melanocytes that are greatly decreased in melanoma cells. Moreover, using immune serum for the ARE-binding protein AUF1, we found decreased AUF1 protein levels in melanoma cells versus melanocytes. The difference in AUF1 expression may account for the longer IL-10 mRNA half-life in melanoma cells versus normal melanocytes, and account for a possible mechanism of deregulation in IL-10 expression in cancer versus normal cells.

20001

ALTERED PHOSPHORYLATION OF AUF1 AND MODULATION OF CYTOKINE mRNA DECAY IN MONOCYTIC CELLS

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Activation of signal transduction pathways in monocytes either by adherence or other extracellular signals induces the robust expression of a battery of cytokines and inflammatory mediators at both the transcriptional and posttranscriptional levels. Included in these activated pathways are selected members of the protein kinase C (PKC) and MAP kinase families. Direct stimulation of PKC using phorbol esters similarly induces cytokine expression in the monocytic leukemia cell line THP-1. These include TNFα and IL-1ß. A major component of the induction is a rapid 6- to 12-fold stabilization of the mRNAs encoding these factors. Both TNFα and IL-1ß mRNAs contain prominent A+U-rich elements (AREs) in their 3'-untranslated regions that target them for rapid decay. An ARE-binding factor, AUF1, forms high affinity, multi-subunit complexes with these AREs *in vitro* involving both protein:RNA and protein:protein interactions. Metabolic labeling and 2-dimensional western analyses indicate that AUF1 exists as a phosphoprotein in THP-1 cells. Upon stimulation of THP-1 cells with phorbol esters, changes in the phosphorylation status of AUF1 occur concomitant with stabilization of TNFα and IL-1ß mRNAs. We evaluated the ARE-binding activity of AUF1 as a consequence of its altered phosphorylation. Recombinant AUF1 is efficiently phosphorylated by PKC *in vitro*. While AUF1 exhibits ARE-binding activity *in vitro* regardless of phosphorylation status, substantial changes in binding cooperativity and complex assembly occurred following phosphorylation by PKC. Since the binding affinity of AUF1 strongly correlates with rapid turnover of ARE-containing mRNAs *in vivo*, modulations in cooperative RNA-binding behavior may contribute to the selective stabilization of target mRNAs.

20002

HuR BINDS THE AU-RICH REGION OF TNF-α mRNA AND ITS OVEREXPRESSION STABILISES A TNF-α mRNA REPORTER

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The regulation of mRNA stability and translation are important mechanisms for the control of TNFα expression in monocytes and macrophages. An AU-rich region (AUR) in the 3' untranslated region of the mRNA (which contains 5 copies of the AUUUA motif) is implicated in both processes. Trans-acting proteins binding to the AUR are thought to confer mRNA instability in resting cells, and to stabilise the mRNA in response to a stimulus such as lipopolysaccharide. The nature of the controlling factors, and the mechanisms involved are unclear, but stabilisation depends upon activation of the p38 MAP kinase pathway. In order to investigate the mechanism of regulation through the AU-rich region we have set out to identify the proteins which bind to the TNF mRNA 3' UTR in RNA band-shift assays. Extracts of RAW 264.7 macrophages contained proteins that formed electrophoretically distinct complexes with a 75 nucleotide probe containing the AU-rich region of the TNF mRNA. An RNA-binding protein responsible for two major complexes was purified and identified as HuR by amino acid sequencing by mass spectrometry. The identification was confirmed by showing that an antibody to HuR supershifted both the mRNA/protein complex bands seen on electrophoresis.

HuR complex formation with the 3' UTR probe was found to be regulated in RAW 264.7 cells by lipopolysaccharide treatment. Overexpression of HuR in a Hela cell tet-off system was shown to stabilise an otherwise unstable reporter construct bearing the AU-rich region of TNF mRNA. The results strongly suggest that HuR is involved in stabilising TNF mRNA during macrophage activation.

20006

The expression of Tristetraprolin is regulated by the mitogen activated protein kinase p38 signal transduction pathway.

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The biosynthesis of TNFα is regulated by the mitogen activated protein kinase (MAPK) p38 and its substrate MAPKAPK-2 (MAPK-activated protein kinase 2). Inhibitors of p38 block TNFα production in LPS-stimulated macrophage cell lines, whilst mice deficient in MAPKAPK-2 display impaired TNFα production following LPS administration. The deletion of a short AU-rich region within the TNFα 3' UTR results in TNFα overexpression, and a loss of sensitivity to p38 inhibitors. This suggests that the regulatory effects of p38 are mediated by the 3' UTR. To determine the mechanism of action of p38, we have set out to identify proteins which bind to the TNFα 3' UTR. This RNA fragment formed several complexes with mouse macrophage RAW264.7 cell extracts, one of which was strongly up-regulated following stimulation of cells with bacterial lipopolysaccharide (LPS). The induction of the complex required *de novo* transcription and translation, and was relatively slow, peaking approximately two hours after the stimulus. The inducible complex contained tristetraprolin (TTP), since it was supershifted in the presence of antibodies against either the C-terminus or N-terminus of TTP. LPS induced both synthesis and phosphorylation of TTP. The synthesis of TTP protein, and the formation of the inducible complex were dependent upon p38. TTP is thought to negatively regulate TNFα gene expression, as the TTP knock-out mouse overproduces TNFα. These observations suggest a complex role for the p38 pathway in the control of TNFα gene expression. Thus p38 positively regulates TNFα biosynthesis, but is also required for the expression of a negative regulator of TNFα biosynthesis.

Cytokines and interferons in transplantation

21005

Effectors mechanisms of allograft rejection: a role for TH2-type responses.

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Acute cellular rejection is usually considered as a TH1-type process involving IFN-γ as a key mediator. On the other hand, it has been proposed that the non-agressive CD4 T cells which are commonly observed in accepted allografts might belong to the TH2 subset. This concept is challenged by several recent studies. Indeed, we developed several models in which IL-5 secretion by TH2 cells results in rejection of allografts. First, we studied the rejection of skin grafts from bm12 mice as donors in Fas-L-deficient gld C57Bl/6 mice as recipients, a model of major histocompatibility complex class II incompatibility in which the recipient is unable to mount anti-donor cytolytic responses. Massive eosinophil infiltrates were found in rejected allografts and their critical role in the rejection process was demonstrated by the prolongation in allograft survival upon IL-5 neutralization The involvement of eosinophils was also demonstrated in acute rejection of heart allografts in the setting of CD8 T cell depletion. In parallel, we found that a short course of anti-CD3 monoclonal antibody in wild-type C57Bl/6 mice grafted with bm12 skin resulted in chronic skin allograft rejection characterized by intense dermal fibrosis, obliterative intimal vasculopathy and leukocytic infiltrates rich in eosinophils. Because these changes occurred in the absence of alloreactive antibodies, we examined the contribution of cytokines in their pathogenesis. Chronically rejected grafts showed a marked accumulation of both IL-4 and IL-5 mRNA. The use of IL-4- or IL-5-deficient mice as recipients allowed to establish the critical role of TH2-type cytokines in this model of chronic rejection. We conclude that TH2-type responses can result in both acute and chronic rejection of allografts. This alternate effector mechanism becomes critical when cytotoxic and TH1-type responses are deficient.

21001

CYTOKINE GENE POLYMORPHISMS IN ORGAN FAILURE AND AFTER ORGAN TRANSPLANTATION

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Cytokines play an important role in host defense mechanisms by regulating a variety of immune and inflammatory responses. In recent years multiple gene polymorphisms have been identified that are related to the level of cytokine and cytokine receptor produced. Therefore, cytokine related pathology could have a genetic background. The availability of genetic markers allowed us to identify patients at risk for cardiomyopathy and allogeneic immune responses after transplantation. A functional single nucleotide polymorphism in exon 1 at position +869 of the anti-inflammatory cytokine TGF-ß gene, a key inhibitor of atherosclerosis, was associated with cardiomyopathy and freedom of transplant coronary disease. Also, susceptibility of our patients to the nephrotoxic effects of the immunosuppressant cyclosporin was related this TGF-ß gene polymorphism. While no clear data are available that the genes containing highly polymorphic dinucleotide repeats or microsatellite markers are associated with the level of cytokine production, also association studies with these genes were performed. Given the prominent role of IL-2 in transplant rejection the dinucleotide repeat in the 3' flanking region of the gene was studied. Analysis revealed that allele 135 is associated with low immune responsiveness after clinical heart transplantation.
In summary, data provided by cytokine gene polymorphisms of *in vivo* case-control studies significantly contribute to our understanding of the pathology of cytokine related diseases including autoimmunity, chronic inflammatory diseases, allergy, and transplant rejection. Because most of these diseases are regulated by a network of cytokines, analysis of gene polymorphism profiles are necessary.

21004

INTERLEUKIN-9 TRIGGERS ACUTE EOSINOPHILIC REJECTION OF HEART ALLOGRAFT IN MICE.

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We recently demonstated that alloreactive Th2 CD4$^+$ T cells can promote the rejection of heart and skin allografts in the mouse. This rejection pathway requires the secretion of IL-5 and involves the recruitment of eosinophils in rejected tissues. We undertook studies to determine the role played by the Th2 lymphokine IL-9 in this new pathway of rejection. Using the Life-PCR technology, we detected high levels of IL-9 mRNA in MHC class II-disparate bm12 skin allografts. To asses the importance of IL-9 in the process of allograft rejection, IL-9 was neutralized *in vivo* by immunizing B6 mice with mouse IL-9 chemically coupled to OVA.High titers anti-IL-9 neutralizing antibodies were detected in these animals at six months after immunization. Controls included mice immunized with OVA alone. *In vivo* neutralization of IL-9 prevented acute rejection of bm12 skin in about 60% of allografted B6 mice and induced long-term graft acceptance in 35% of recipients (survival >40 days). All the graft performed on control animals were acutely rejected (survival ≤12 days). These experiments demonstrate the prime role played by IL-9 in the rejection of MHC class II-disparate skin allografts. To investigate the effector mechanisms of rejection promoted by IL-9, we analyzed the effect of local expression of IL-9 on allograft survival. IL-9 transgenic expression in cardiac bm12 grafts induces acute rejection in normal B6 recipients (survival <30 days compared to non-transgenic control grafts and transgenic isografts with survival >50 days). This IL-9-mediated rejection was characterized by dense eosinophilic infiltration, acute vasculitis and fibrosis. Treatment with anti-IL-5 neutralizing antibodies prevented eosinophilic infiltration and extended graft survival (>50 days). Administration of anti-IL-4 neutralizing antibodies fully prevented rejection of IL-9-expressing cardiac grafts. Our study identifies IL-9 as an essential factor for allograft rejection mediated by Th2 CD4$^+$ T cells.

21006

THE FREQUENCY OF IL-2 PRODUCING T-LYMPHOCYTES PREDICTS ACUTE REJECTION AFTER TRANSPLANTATION EVEN BEFORE TRANSPLANTATION

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Recently, we found that during acute rejection (AR) the number of IL-2 producing T-helper lymphocytes increased. We questioned whether the frequency of IL-2 producing T-cells before transplantation is predictive for AR after kidney transplantation and whether it can predict AR after conversion in immunosuppressive therapy. One year after transplantation patients were converted from cyclosporine A (CsA) to azathioprine (AZA) or mycophenolate mofetil (MMF). We measured the frequency of donor and third-party reactive IL-2 producing cells in peripheral blood before transplantation and prior to conversion using limiting dilution assays.
Patients who experienced AR in the first posttransplant year had higher numbers of donor-specific IL-2 producing cells before transplantation than patients who remained free from AR (n=37: median 194/10^6 vs. 62/10^6 PBMC; p=0.009). Moreover, in contrast to patients with AR, only patients without AR had undetectable frequencies (<10/10^6 PBMC: 0/13 vs. 7/24; p=0.04). The frequency of donor-specific IL-2 producing cells before conversion was not predictive for AR after conversion (n=30; -AR: 37/10^6 vs. +AR: 50/10^6 PBMC; p=0.85). The number of third-party reactive IL-2 producing cells did not correlate with AR.
In conclusion, undetectable numbers of donor-specific IL-2 producing T-lymphocytes measured before transplantation are predictive for a rejection-free first year after transplantation.

21007

CONVERSION FROM CYCLOSPORINE TO TACROLIMUS DOWN REGULATES THE TGF-β SYSTEM AND IMPROVES RENAL FUNCTION, CHOLESTEROL LEVELS AND BLOOD PRESSURE IN HEART TRANSPLANT RECIPIENTS

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Transforming growth factor β_1 (TGF-β_1) is a multifunctional protein with immuno modulatory and fibrinogenic properties. It is thought to be resposible for the development of graft sclerosis, lipid abnormalities, and hypertension in cyclosporine (CsA)-treated cardiac allograft recipients. The immunosuppressant CsA is known to enhance the TGF-β concentration, whereas tacrolimus (Tac) does not affect TGF-β plasma levels To evaluate the effects of CsA and Tac on the TGF-β system in cardiac allograft recipients, we studied the expression levels of receptors of the TGF-β system, Type1 and Type2, on lymphocytes and monocytes, TGF-β mRNA expression in endomyocardial biopsies, before and after conversion from CsA to Tac in 10 stable heart transplant patients. Results: no rejection grade >1 (ISHLT criteria) was seen after conversion. Diastolic and systolic blood pressures decreased significantly, (p=0.03 and 0.01, respectively), as well as serum cholesterol levels: from 6.0 mmol/l to 5.3 mmol/l, p=0.02. Serum creatinine decreased from 145µmol/l to126µmol/l, n.s. The receptor expression of TGF-βR1 decreased on both lymphocytes and monocytes, while the expression of TGF-βR2 was not influenced. The intragraft mRNA expression for TGF-β_1 was comparable before and after conversion. We conclude that conversion from CsA to Tac in heart transplant recipients results in a downregulation of the TGF-β system, reflected by a decreased peripheral TGF-β receptor expression, resulting in improvement of cholesterol levels and blood pressure.

21008

RENAL FAILURE AFTER CLINICAL HEART TRANSPLANTATION IS ASSOCIATED WITH THE TGF-ß1 (CODON 10) GENE POLYMORPHISM

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To determine whether genetic factors are involved in the development of renal dysfunction due to cyclosporin nephrotoxicity we analyzed two functional polymorphisms in the signal sequence of the transforming growth factor (TGF)-ß1 gene; codon 10 ($Leu^{10} \rightarrow Pro$) and codon 25 ($Arg^{25} \rightarrow Pro$). Using sequence specific oligonucleotide probing both TGF-ß1 gene polymorphisms were analyzed in cardiac allograft recipients (n=168) who survived at least 1 year with a minimal follow-up of 7-years. Patients received cyclosporin and steroids as maintenance immunosuppressive therapy. Renal dysfunction was defined as a serum creatinine ≥ 250 µmol/l. Renal dysfunction was observed in 2% (3/168) of the patients at 1-year, in 7% (11/160) at 3-year, in 12% (18/152) at 5-year, and in 20% (26/131) at 7-year post-transplantation.The genotypic distributions for TGF-ß1 codon 10 were:7% Pro/Pro, 61% Pro/Leu, and 32% Leu/Leu, and for codon 25 these percentages were 1% Pro/Pro, 12% Pro/Arg, and 87% Arg/Arg. An association was found between the TGF-ß1 genotype encoding proline at codon 10 and renal dysfunction. At 7-years post-transplantation, 26% (23/89) of the patients with the heterozygous Pro/Leu or homozygous Pro/Pro genotype had renal dysfunction vs only 7% (3/42) of the patients with the homozygous Leu/Leu genotype (p=0.017). For the TGF-ß1 codon 25 genotypes no association was found between TGF-ß1 genotypes and renal dysfunction.Our data support the hypothesis that TGF-ß1 is involved in the process leading to renal insufficiency in cyclosporin treated cardiac allograft recipients. In these patients the presence of TGF-ß1 Pro^{10} might be a risk factor.

21002

ELEVATED IL-18 AND IL-18 BP LEVELS IN ACUTE GVHD POST ALLO SCT.

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IL-18 is an inflammatory cytokine, which strongly induces IFNγ and regulates cytotoxicity, while IL-18 BP inhibits its functions. Graft versus host disease (GVHD) represents a major obstacle in successful outcome of allogeneic peripheral blood stem cell transplantation (allo SCT). We have previously shown that increased inflammatory cytokines (IL-1ß,IL-6,TNFα) as well as IFNγ/sIFNγR play a central role in the inflammatory and allospecific components of GVHD post- allo SCT. Therefore we presently evaluated IL-18/IL-18 BP levels pre and post-allo SCT in 21 consecutive pts with hematological malignancies. 10 pts developed acute GVHD and 2 pts rejected their graft . 9 pts underwent uneventful allo SCT. The levels of IL-18 were found to be markedly elevated in all GVHD pts (315pg/ml), while lower and similar levels were found in pre allo SCT (96pg/ml), post allo SCT (85pg/ml), following engraftment (118pg/ml) and during rejection (82pg/ml), which were all significantly different ($p<0.05$) than normal controls (40pg/ml). Increasing levels of IL-18 were prognostic of GVHD development. IL-18 BP increased in acute GVHD (15.1ng/ml) in parallel to sIFNγR, declined (4.2ng/ml) during conditioning and increased in direct correlation with engrafment (9.9ng/ml). In vivo experiments testing the effect of anti IL-18 Ab in a GVHD model, are performed. In conclusion, most pts with alloSCT displayed high levels of IL-18/IL-18 BP mediating increases in IFNγ/sIFNγR reaching maximal levels in GVHD. IL-18/IL-18BP may therefore be used as diagnostic tools in identification and early detection of GVHD after allo SCT. enabling earlier treatment.

21011

MOLECULAR CLONING OF PORCINE FAS-LIGAND AND CASPASE-3; THEIR RELATION WITH APOPTOSIS OF PORCINE PK-15 CELLS.

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Fas/Fas-ligand interaction and Caspase cascade are important signals of apoptotic cell death in several infectious diseases, immune reactions and xenotransplantation. We isolated cDNAs contained the coding sequence of porcine Fas-ligand (Fas-L) and Caspase-3 (Casp-3). Total-RNA prepared from porcine thymocyte stimulated with 5 µg/ml concanavalin A (ConA), alveolar macrophages stimulated with 10 µg/ml lipopolysaccharide (LPS) was used to clone the cDNA of porcine Fas-L and Casp-3 by reverse transcription polymerase chain reaction (RT-PCR), respectively. The open reading frame (ORF) of the porcine Fas-L cDNA is 849 base pairs (bp) in length and encodes 282 amino acids. which is 85.5%, 76.6% and 75.5% homologous to the predicted human, murine and rat Fas-L, respectively. The ORF of the porcine Casp-3 cDNA is 834 bp in length and encodes 277 amino acids, which is 88.4%, 86.6% and 87.7% homologous to the predicted human, murine and rat Casp-3, respectively. The mRNA expression of porcine Fas was detected in porcine PK-15 cells by RT-PCR analysis. The recombinant porcine Fas-L expressed by a baculovirus system induced apoptosis to PK-15 cells sensitized by cycloheximide (CHX). Moreover, the enzymatic activity of Casp-3 in PK-15 cells after Fas-L stimulation was significantly increased, but not Caspase-1 activity. The neutralizing anti-human Fas monoclonal antibody (clone ZB4) was able to inhibit the apoptotic cell death of PK15 cells by Fas-L. However, the anti-porcine TNF-α monoclonal antibody (clone 4F4) and the inhibition of Casp-3 by its specific inhibitor were not able to prevent the apoptotic cell death of PK15 cells by Fas-L. These results described here will be useful for further investigation of these molecules in porcine apoptotic cell death.

21010

INTRAGRAFT IL-2 mRNA EXPRESSION DURING ACUTE REJECTION IS ASSOCIATED WITH INCREASED TOTAL WALL THICKNESS AFTER CLINICAL HEART TRANSPLANTATION

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Interleukin-2 (IL-2) is an important cytokine in the regulation of acute rejection after transplantation. To determine whether local IL-2 expression is associated with dysfunction of transplanted hearts, we monitored 15 heart allograft recipients during the first three months after transplantation. The presence of IL-2 mRNA in heart biopsies (n = 123) was determined by RT-PCR. To measure heart function, echocardiograms, recorded within 8 hours of biopsy sampling, were analysed for the following parameters: total wall thickness (TWT), mitral valve early and atrial diastolic velocity, E/A ratio, deceleration time and isovolumetric relaxation period. Data were related to histological signs of acute rejection (AR), characterized by aggressive inflammatory infiltrates. Histological AR was strongly associated with local IL-2 mRNA expression ($p = 0.002$, Fischer's exact test). No relation was found between either histology or IL-2 mRNA expression and the studied echocardiographic parameters. However, after stratifying our data into those with and without AR, we found that IL-2 mRNA expression during AR was significantly associated with increasing TWT prior to AR ($p = 0.048$, Fischer's exact test). Patients with IL-2 mRNA expression during their first AR showed overall increasing TWT before AR (mean +0.22 cm ± 0.12) versus none of the patients without IL-2 mRNA expression during their first AR (mean -0.18 cm ± 0.12). Four non-rejecting controls showed decreasing TWT before their first IL-2 positive EMB (mean -0.30 cm ± 0.31). In summary, our results show significantly increased total wall thickness prior to IL-2 positive acute rejection. We conclude that rejection accompanied by intragraft IL-2 mRNA expression has a greater influence on heart morphology and therefore may be indicative for more severe rejection.

21012

FREQUENCIES OF IL-2 PRODUCING CELLS IN PERIPHERAL BLOOD REFLECT DONOR DIRECTED REACTIVITY IN THE GRAFT AFTER CLINICAL HEART TRANSPLANTATION.

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For the diagnosis of acute rejection after heart transplantation endomyocardial biopsies are performed on a routine base. Determination of the frequency of alloreactive Il-2 producing T Helper Lymphocytes or Cytotoxic T Lymphocytes in peripheral blood could give an indication whether these EMB are really needed. However the methods generally used take to much time to be of clinical relevance. A LDA for CTLp frequency measurements takes 7 to 10 days and for HTLf 4 days. We developed a fast (48 hour) limiting dilution assay for the enumeration of allo-reactive Helper T Lymphocytes in the peripheral blood of heart transplant recipients. HTLf were related to the histological scores of the endomyocardial biopsies. HTLf in pre-transplant samples varied from patient to patient, ranging from 106 to 625 HTL/10^6 PBMC. In the first week after HTx when immunosuppression was instituted HTLf were significant lower (range 30 - 190 HTL/10^6). The level of HTL in the first week after HTx when rejection grade was 0 or 1A (ISHLT score) was considered to be the baseline frequency. This frequency did not correlate with the number of subsequent rejection episodes. During rejection (grade 3), donor-specific HTLf were increased above their baseline frequencies ($p= 0.01$). Expressed as proportion of baseline frequencies, HTLf significantly increased during AR ($p= 0.003$). The increase was specific, since viral infections did not result in a rise of donor specific HTL, while HTLf specific for third party HLA-antigens did not rise during rejection. Monitoring HTLf in peripheral blood with a shortened (48 hour) assay may serve as a non-invasive method for detecting intra graft immunological reactivity. Demonstrating absence of donor specific reactivity may limit the number of invasive EMB procedures and allow tapering of immunosuppression.

21013

Branched O-glycans selectively inhibit myeloid cell development

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Core 2 glycosyltransferase (C2GnT), a member of the O-glycan β1,6 N-acetylglucosaminyl-transferase family, is responsible for the branching of O-linked oligosaccharide side chains enabling the formation of lactosamine and polylactosamine antennae on O-glycosylated cell surface molecules including CD34, CD43, CD44, CD45 and PSGL-1. Previously we have shown in transplantation experiments that C2GnT overexpression in transfected bone marrow prevents *in vivo* development of IL-3/GM-CSF responsive myeloid cells, whereas T cell development is not affected. Furthermore, myeloid development was not rescued by 10 day IL-3 treatment of bone marrow recipient mice prior to CFC analysis. We have extended these analyses using a newly developed bicistronic vector encoding C2GnT and GFP. Transfected bone marrow cells from $CD43^+$ donor mice were purified by FACS sorting for GFP^+ cells and transplanted into lethally irradiated (1200Gy) $CD43^{null}$ recipient mice. Two months after transplantation of control (GFP only) vector transfected bone marrow we were able to detect donor derived $CD43^+$ T cells, B cells and myeloid cells, whereas donor derived myeloid cells were again absent in recipients of C2GnT-GFP transfected bone marrow recipient, confirming our earlier data that increased O-glycan branching inhibits in vivo myeloid cell development. At present the mechanism responsible for the selective effects of C2GnT overexpression on myelopoiesis are not know. We are currently evaluating the role of cytokines in regulation of C2GnT expression in myeloid cells.

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21009

TREATMENT WITH AN IL-2 RECEPTOR ANTAGONIST AFFECTS BOTH THE IL-2 AND IL-15 SIGNALLING PATHWAYS AFTER CLINICAL KIDNEY TRANSPLANTATION

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Recently it became evident that targeting the IL-2/IL-2R pathway with humanized or chimeric anti-IL-2Rα (CD25) antibodies, in combination with cyclosporin and mycophenolate mofetil significantly reduces the incidence of acute rejection after clinical organ transplantation. However, due to redundancy in the cytokine network, patients may still reject their allograft as free β and γ-chains of the IL-2R complex could bind alternative T-cell factors (IL-7, IL-15). We have studied whether treatment with the chimeric anti-IL-2Rα basiliximab (CD25) is associated with unimpaired expression of the β-chain (CD122) and γ-chain (CD132) of the IL-2R complex. By flowcytometry the IL-2R complex was analysed in pre- and posttransplant peripheral blood samples from renal allograft recipients (n=25) who received basiliximab (20 mg IV bolus on day 0 and 4), cyclosporin, mycophenolate mofetil, and a 3-day course of steroids. In peripheral blood, after induction therapy with basiliximab, no CD25 positive T-cells were detectable for 49 days (median, range 25-69), indicating that anti-CD25 mAb binds to the IL-2Rα. At these points in time, when CD25 cells were covered with basiliximab, the mean fluorescence intensity (MFI) of IL-2Rß (CD122) positive T-cells significantly decreased. The IL-2Rβ MFI counts fell by a factor 2-3 ($p=0.01$), while the MFI of IL-2Rγ (CD132) positive T-cells remained stable. Functional analysis of these CD25 negative cells confirmed that indeed the responsiveness on IL-15 is impaired. During anti-CD25 treatment the IL-15 driven proliferation is inhibited with 30 percent. Our results show that apart from IL-2Rα (CD25) blockade, basiliximab has an additional effect on the expression of IL-2Rß (CD122). Thus, therapy with basiliximab may prevent acute rejection not only by blocking IL-2 signalling but also by impairing IL-15 signalling through down-regulating the IL-2/IL-15 receptor β-chain.

Mode of action of cytokines I

22003

THE BIMODAL EFFECT OF ENDOGENOUS IFN-γ IN MURINE MODELS OF AUTOIMMUNE DISEASE: A ROLE FOR MYCOBACTERIAL ADJUVANT-INDUCED MYELOPOIESIS.

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Functional ablation of IFN-γ by antibody or by gene knock-out has revealed a bimodal role of endogenous IFN-γ in the pathogenesis of murine experimental autoimmune disease models. Depending on the model, the overall role of IFN-γ appeared to be either disease-promoting (myasthenia gravis, orchitis, lupus and diabetes) or -limiting (encephalomyelitis, uveitis and nephritis). Alternating effects, depending on the experimental conditions imposed, were seen in autoimmune thyroiditis and in collagen-induced arthritis (CIA). Immunostimulatory (e.g. MHC expression) and proinflammatory (e.g. macrophage activation) actions of IFN-γ have been amply documented and provide a plausible conceptual framework to explain disease-promoting effects of IFN-γ. Contrastingly, mechanisms for autoimmune disease-inhibitory roles of IFN-γ have received much less attention, e.g. apoptosis of auto-antigen-reactive T cells, induction of suppressor cells, altered pattern of chemokine production. Studies on CIA have provided a new clue. If induced by immunisation with collagen II in mycobacteria-containing complete Freund's adjuvant (CFA), the effect of IFN-γ ablation is to make joint involvement worse. Contrastingly, if incomplete adjuvant is used, the effect is to reduce disease severity. A correlate of these opposing effects is enhanced or decreased cutaneous DTH against the autoantigen. Significantly, the use of CFA is associated with days-long augmentation of myelopoiesis, resulting in increased numbers of Mac-1+ cells, representing immature neutrophils and monocytes. In IFN-γ-ablated mice this myelopoietic effect of CFA is much more pronounced, indicating that in normal mice it is kept in check by endogenous IFN-γ. That the Mac-1+ cell population is a crucial player in CIA pathogenesis is suggested by coincidence of the myelopoietic peak with onset of disease, and by experiments showing that treatment with antibodies against IL-6 or IL-12 results in inhibition of both the myelopoietic burst and the disease. Inhibition myelopoiesis by IFN-γ may explain disease inhibitory effects of IFN-γ in other autoimmune disease models which rely on the use of CFA.

22008

Interleukin-6 modulates Interferon-regulated gene expression by inducing the ISGF3γ gene using CCAAT/Enhancer Binding Protein-beta (C/EBP-β)

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Interluekin-6 and IFNs are potent regulators of cell growth and immune responses. Although IL-6 alone does not induce the expression of IFN-stimulated genes (ISG), a low dose priming of cells with IL-6, strongly enhances the cellular responses to IFN-α. This effect of IL-6 is not due to super stimulation of JAK-STAT pathway. Rather, IL-6 induces expression of ISGF3γ (p48), a sub-unit of multimeric transcription factor ISGF3. As a result, IFN-α robustly activates gene transcription in IL-6 primed cells. We have shown earlier that the transcription of ISGF3γ gene is regulated through a novel element GATE (gamma-IFN activated transcriptional element). We show here IL-6 induces the ISGF3γ gene through GATE. Transcription factor C/EBP-β is required for inducing the ISGF3γ gene expression through GATE. A mutant c/EBP-β inhibits the IL-6 inducible ISGF3γ gene expression through GATE. Together, these results establish a molecular basis for the synergy between IFNs and IL-6.

22010

Identification and characterization of the novel Genes associated with Retinoic acid-Interferon induced Mortality (GRIM), using a genetic approach: Mechanism of action and Role in tumor cell growth suppression by cytokines

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Interferons (IFN) are prototypic growth suppressive cytokines. However, they have limited effects on the growth of solid tumors. Retinoids, a group of Vitamin A related compounds, inhibit the growth of certain tumor cells. We show that IFN-β/Retinoid combination causes breast tumor cell death in vitro and in vivo. To understand the molecular basis for these actions, we have employed the antisense technical knock-out strategy and identified the Genes associated with Retinoid-IFN induced Mortality (GRIM). One such gene, GRIM-12, is identical to redox enzyme thioredoxin reductase (TR). Inhibition of TR confers growth advantage to cells in the presence of IFN/RA. Over expression of TR increases the cellular susceptibility to IFN/RA induced death. TR is required for the Caspase activation and the functional activity of p53 tumor suppressor. Mutant TR interferes with the death activating actions of TR by suppressing Caspase-8 activation in response to IIFN/RA or TNF-α treatment. Similarly the substrate of TR, thioredoxin is also required for cytokine induced death pathways. Two other novel genes GRIM-1 and GRIM-19 also mediate IFN/RA induced cell death. GRIM1 encodes a family of novel proteins involved in cell death. Inhibition of GRIM-1 by antisense imparts resistance to IFN/RA induced cell death. Over expression of GRIM-1 causes nuclear fragmentation and cell death. Death activation by GRIM-1 is dependent on caspases. GRIM-19 encodes a nuclear protein that causes cell death in response to IFN/RA combination. Together, our studies identified novel pathways and potent mediators of IFN/RA induced death. These data have important implications for tumor cell therapy with cytokines and may help develop new combination therapies.

10010

IL-7 stimulates tyrosine phosphorylation of clathrin which is constitutively associated with IL-7Rα chain.

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Tyrosine phosphorylated proteins induced by IL-7 stimulation were investigated in an IL-7-dependent thymocyte line, D1. IL-7 stimulation rapidly induced phosphorylation of a protein of approximately 200kd. This phosphoprotein was purified by anti-phosphotyrosine affinity, HPLC and gel filtration. Using mass spectroscopy a sequence was obtained corresponding to clathrin heavy chain. Using immunoprecipitation with anti-clathrin antibodies, we verified that clathrin was heavily phosphorylated within three minutes following IL-7 stimulation of D1 cells or normal pro-T cells. Phosphorylation was blocked by inhibitors of Jak3. Of the cytokines using the γc family of receptors, IL-7 induced the most dramatic clathrin phosphorylation, IL-4 somewhat less, and none was observed for IL-2, -9 or -15. Unlike other signaling receptors, such as the IL-2Rβ chain, with which clathrin associated following ligation, clathrin was associated with IL-7Rα prior to ligand stimulation as demonstrated by co-immunoprecipitation. Clathrin is normally involved in internalization of signaling receptors following ligation, whereas constitutive association is more characteristic of transport receptors that are continuously internalized. The constitutive association of clathrin with IL-7Rα apparently explains the extremely rapid, ATP-dependent internalization of this chain following antibody crosslinking. Using confocal microscopy, internalized IL-7Rα was visualized in endosomes. The ability of IL-7 stimulation to phosphorylate clathrin may influence the fate of internalized IL-7α chains since clathrin can direct vesicles either to degradation or recycling pathways.

22002

PIVOTAL ROLE OF IRF-1 TRANSCRIPTION FACTOR IN G-CSF-INDUCED GRANULOCYTIC DIFFERENTIATION

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Biological responses to cytokines are mediated by intracellular pathways involving induction of signaling and metabolic cascades. G-CSF, a regulator of granulopoiesis, is critical for directing normal neutrophil production and maturation. Interferon regulatory factor-1 (IRF-1) and its functional antagonist IRF-2 are cytokine-inducible transcriptional regulators involved in the regulation of hematopoiesis, immune response, cell proliferation and differentiation. In the present study, we investigated mechanisms underlying the differentiation inducing properties of G-CSF using the murine 32D Cl3 cell line as a model of granulopoiesis. Upon G-CSF treatment, specific transcriptional stimulation of IRF-1 was observed; IRF-1 acted both in growth arrest and in cooperation with G-CSF to induce transcription of specific markers of granulocytic differentiation. The pivotal role of IRF-1 in the process of granulocytic differentiation was demonstrated in 32D Cl3 cells stably expressing IRF-1; these cells displayed an accelerated onset of growth arrest and acquisition of the differentiated phenotype following G-CSF treatment, as compared to control cells. Conversely, constitutive expression of IRF-2 completely abolished neutrophil maturation. Moreover, using the STAT binding element present in the IRF-1 promoter, we demonstrated that binding of STAT5 and STAT1 was activated by the G-CSF treatment. Together these studies point to a hierarchy of transcriptional steps during G-CSF-induced neutrophilic differentiation of 32D cells that involves specific STAT(s) and IRF-1 as limiting components of the differentiation pathway.

22004

IDENTIFICATION OF NOVEL IL-1β-INDUCED GENES IN PANCREATIC β-CELLS BY HIGH DENSITY OLIGONUCLEOTIDE ARRAYS

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Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease resulting from the selective destruction of insulin-producing pancreatic β-cells. IL-1β may contribute to pancreatic β-cell death in T1DM. Prolonged (24-72h) β-cell exposure to IL-1β induces cell dysfunction, while culture in the presence of IL-1β + INFγ for 3-9 days leads to apoptosis. To clarify the mechanisms involved in these effects of IL-1β, we studied the general pattern of IL-1β-induced genes in β-cells. Primary rat β-cells were FACS-purified and exposed for 24 h to control condition or to IL-1β (50 U/ml). A pool of 1.4 x 10^6 cells obtained from 6 separate cell isolations was used for each condition. The gene expression profile was analyzed by a high-density oligonucleotide array ("GeneChip", Affymetrix) representing 5000 full-length genes + 3000 EST's. The expression of some genes of interest was confirmed by RT-PCR. Nearly 2000 genes or ESTs were detected in control and IL-1β-treated β-cells. Of these, nearly 130 displayed a greater than 3-fold increase or decrease in expression following IL-1β exposure. From the 16 β-cell genes or proteins previously described as modified by IL-1β, 13 were detected in the present analysis, confirming the sensitivity of the method. These genes are: arginase (decreased, D), iNOS (increased, I), ornithine decarboxylase (I), argininosuccinate synthetase (I), ICAM-1 (I), insulin (D), glucokinase (D), cholecystokin-A receptor (D), heat shock protein 70 (I), Mn superoxide dismutase (I), CINC-1 (I), MCP-1 (I) and IRF-1 (I). The 85 genes modified by IL-1β included hormone and cytokine receptors, chemokines, proteins related to arginine metabolism and NO production, ionic channels, proteins potentially related to β-cell function and/or glucose metabolism, oncogenes and transcription factors. In conclusion, IL-1β induces a complex pattern of gene expression in pancreatic β-cells. Clarification of the function and regulation of these "IL-1β-induced gene patterns", rather than that of individual genes, may allow us to understand how IL-1β contributes to β-cell dysfunction and death in T1DM.

22009

HYPOXIA-INDUCIBLE FACTOR 1, A NOVEL TRANSCRIPTIONAL MEDIATOR OF IL-1 AND TNF-α EFFECTS

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Hypoxia-inducible factor 1 (HIF-1) is a heterodimeric transcription factor composed of the O_2-labile α- and the constitutive β-subunit. HIF-1 controls gene expression of proteins involved in the adaptation to reduced O_2-availability, such as erythropoietin (EPO), vascular endothelial growth factor (VEGF), inducible NO synthase and glucose transporter 1. Because the expression of several of these genes is altered in inflammation, we investigated the effects of IL-1β and TNF-α on the activation of the HIF-1 DNA-binding complex and the amount of HIF-1α protein in human hepatoma (HepG2) and primary renal proximal tubular epithelial cells (PTEC) in culture. These cell lines were chosen, because they produce EPO (HepG2) and VEGF (HepG2, PTEC) in a pO_2-dependent manner. Under normoxic conditions, both cytokines caused a moderate activation of HIF-1 DNA-binding in HepG2 cells. In hypoxia, cytokines strongly increased HIF-1 activity compared with the effect of hypoxia alone. Since only IL-1β increased HIF-1α protein levels in HepG2 cells, the effect of this cytokine was studied in more detail in PTEC. IL-1β increased HIF-1α protein levels both in normoxic and hypoxic PTEC. This effect was completely abolished by phosphatidylinositol 3-kinase inhibitor LY294002. The MAP-kinase inhibitor PD98059 was ineffective. Thus, increased HIF-1 DNA-binding to hypoxia-responsive elements may partly account for the stimulation of the production of proinflammatory mediators such as VEGF and NO not only on exposure to hypoxic stress but also to IL-1β and TNF-α. We propose that HIF-1 is critically involved in modulating gene expression during inflammation.

22026

IL-1α, IL-1β AND IL-1Ra ARE DIFFERENTLY EXPRESSED IN TESTICULAR CELLS OF IMMATURE MICE UNDER *IN VIVO* AND *IN VITRO* CONDITIONS.

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IL-1 activity was detected in testicular homogenates and was shown to affect physiological functions of testicular cells such as testosterone secretion by Leydig cells and transferrin secretion by Sertoli cells.

The aim of the present study was to characterize the cellular source(s) and the expression levels of IL-1α, IL-1β and IL-1 receptor antagonist (IL-1ra) in the testicular cells of immature mice under normal and pathological conditions in vivo and in vitro.

Immature mice (2 weeks) were injected (i.p.) with saline or LPS (10μg/mouse). 4, 16 and 24 hours later, testicular tissues were fixed in formalin for immunohistochemical staining (IHC) or were processed (from normal mice) for Sertoli cell isolation. Purified Sertoli cells were cultured and stimulated with LPS (5 μg/ml) for 4-24 hours. Fixed testicular tissues and Sertoli cell cultures were examined for IL-1α, IL-1β and IL-1ra expression levels by IHC and specific ELISA kits. Our results (IHC) show that IL-1α, IL-1β and IL-1ra were expressed in Sertoli Cells, Germ cells and Leydig cells. IL-1β expression was lower than IL-1α and IL-1ra in these tissues. IL-1ra was also expressed in Golgi apparatus of mainly Sertoli cells. LPS injection (i.p) to the mice did not affect the expression levels of IL-1α, IL-1β and IL-1ra in the testicular tissue cells. Cultures of purified Sertoli cells showed (by IHC and ELISA) basal expression levels of IL-1α and IL-1ra only in the lysate of the cells (production). Stimulation of Sertoli cell cultures with LPS significantly increased their capacity to produce IL-1α after 2-24 hours of stimulation and IL-1ra after 8-24 hours. However, IL-1β could not be detected in Sertoli cell cultures under in vitro stimulatory conditions.

Our results show different expression of IL-α, IL-1β and IL-1ra in Sertoli cells under in vivo and in vitro conditions. Also, under in vitro conditions IL-1 activity window seems within 6 hours after stimulation with LPS.

The results presented here may suggest the possibility of involvement of IL-1 system in the physiological as well as pathological functions of testicular cells and the process of spermatogenesis.

Thus, IL-1 system may be involved in the regulation of male fertility.

22033

Ceramides may mediate TNF-α-induced increases of IL-6 mRNA in adipocytes

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Adipose tissue produces several proinflammatory mediators such as TNF-α and IL-6. Adipose tissue production of these cytokines is elevated in obese animals and humans. Indeed, it has been shown that obese individuals have elevated levels of circulating IL-6. IL-6 may induce C-reactive protein synthesis, which is also elevated in obese persons. That suggests the presence of systemic inflammation and the potential for obesity-related complications, such as coronary heart disease. Because TNF-α has been shown to enhance IL-6 production, we have initiated studies to examine the pathways that transduce TNF-stimulated IL-6 production in 3T3-L1 adipocytes. In the present study, 3T3-L1 adipocytes were incubated with TNF-α for 3-12 h and different inducers or inhibitors of PKA and PKC, or with sphingomyelinase; steady-state levels of IL-6 mRNA were then assessed by quantitative kinetic RT-PCR. Intracellular ceramide levels were determined following a 30 minute exposure to TNF-α. TNF-α dose-dependently increased IL-6 message up to 12 h. Although forskolin and dibutyrl cAMP significantly increased IL-6 mRNA levels after 6 h, preincubation with H89, a specific inhibitor of PKA, failed to inhibit the TNF-induced increase of IL-6 message. Preincubation of adipocytes with bisindolylmalemide, a specific inhibitor of PKC, or preexposure to PMA for 16 h, which down-regulates PKC, failed to inhibit the stimulatory effect of TNF-α; analogs of diacylglycerol that activate PKC were unable to mimic the effect of TNF-α. In contrast, sphingomyelinase increased IL-6 mRNA and also enhanced the stimulatory effect of TNF-α. In addition, TNF-α dose-dependently increased ceramide-levels after 30 minutes. Thus, the TNF-stimulated increase of IL-6 mRNA may be mediated by ceramides but not PKA or PKC. Further investigation is required to determine the downstream targets of ceramides that transduce the stimulatory effect of TNF-α. (Supported by NIH Grant DK09950 and the University of California Breast Cancer Research Program)

22032

ANTI-TNF TREATMENT DECREASES APOPTOSIS OF GUT EPITHELIAL CELLS: A NOVEL MECHANISM OF ANTI-TNF THERAPY IN EXPERIMENTAL CROHN'S DISEASE (CD)

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TNF has the ability to induce programmed cell death (apoptosis) directly through receptor-ligand interaction, and may also effect apoptosis indirectly by modulating Fas antigen expression. TNF plays a central role in mediating gut inflammation characteristic of CD, and anti-TNF treatment is highly effective in reducing the severity of disease in patients with CD. However, the precise mechanism(s) of action of anti-TNF administration in CD is unknown. We investigated the role of anti-TNF therapy in protecting intestinal epithelial cells (IEC) from apoptosis in the SAMP1/Yit mouse model of intestinal inflammation, which spontaneously develops an ileitis closely resembling human CD. SAMP1/Yit mice were treated with a single dose of anti-TNF (0.1 mg, n=5; 1.0 mg, n=8) or isotype control (n=8) antibody, and sacrificed after one week. Full-thickness ileal samples were processed for histological analysis, and (IEC) freshly isolated from ileal tissues were stained with propidium iodide (apoptosis marker) or a monoclonal antibody against Fas for FACS analysis. Our results showed that anti-TNF therapy effectively reduced intestinal inflammation in SAMP1/Yit mice (inflammatory index: 7.3 ± 0.2 for 0.1 mg, n.s. and 5.1 ± 0.1 for 1 mg, $p<0.01$ vs. 9.9 ± 0.1 for control). Furthermore, anti-TNF treatment decreased IEC apoptosis (31% for 0.1 mg and 55% for 1.0 mg vs. 68% for control) and Fas expression (35% for 0.1 mg and 53% for 1.0 mg vs. 80% for control). These results further support the important role of TNF in mediating CD, and suggest a novel mechanism by which anti-TNF therapy ameliorates chronic intestinal inflammation in patients with CD.

22031

ABC1-TRANSPORTER INHIBITOR SUPPRESSES IL-1 SECRETION: A WAY TO DOWNREGULATE INFLAMMATION?

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Both IL-1α and IL-1β lack a signal peptide that targets proteins to the classical secretory pathway through ER and Golgi. Alternatively, IL-1 may be translocated at the plasma membrane by transporter proteins of the so-called ATP-binding cassette (ABC) transporter family. Glybenclamide, an ABC1-transporter inhibitor, has been shown to inhibit IL-1β secretion from macrophages. Fibroblasts express IL-1β upon exposure to pro-inflammatory stimuli, such as LPS, or TNF-α. In IL-1β expressing fibroblasts, IL-1 serves to stimulate cells in an autocrine and paracrine fashion, inducing other cytokines and chemokines, and above all the prototypic chemokine IL-8. We hypothesized that glybenclamide, via inhibition of IL-1β secretion, disabled the IL-1 mediated autocrine activation loop, thus suppressing IL-8 induction. Glybenclamide dose-dependently inhibited IL-8 in IL-1β-transfected primary human dermal fibroblasts, as well as in cells that were activated with TNF-α. Glybenclamide at 100 μM suppressed IL-8 to approximately 50%, as measured using ELISA. Glybenclamide also was able to inhibit IL-1α and IL-1β secretion in overexpressing transfected COS cells. We conclude that inhibition of IL-1 externalisation can downregulate inflammation in tissues via suppression of IL-8 induction. Through this pathway, ABC1 transporter inhibitors may constitute a new class of anti-inflammatory drugs.

22029

IFN-γR2 EXPRESSION LEVELS CONTROL ANTI-EMCV ACTIVITY IN HAMSTER CELLS EXPRESSING RECONSTITUTED HUMAN IFN-γ RECEPTOR COMPLEXES

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It has been reported that coexpression of both human IFN-γ receptor chains in either murine or hamster cells did not consistently reconstitute antiviral activity in response to Hu-IFN-γ. In evaluating these observations, we tested the possibility that increased expression of Hu-IFN-γR2 inhibits antiviral activity of Hu-IFN-γ in murine and hamster cells. We expressed Hu-IFN-γR2 under its genomic promoter (gR2), and compared IFN-γ-induced activities to those of cells expressing Hu-IFN-γR2 driven by the cytomegalovirus immediate-early (CMV) and human EF-1α (EF) promoters. Quantitation of Northern blots indicated the expression of Hu-IFN-γR2 driven by the gR2 promoter was one tenth that of CMV-driven IFN-γR2 expression and one-sixtieth that of EF-driven expression. Western blots and fluorescence studies indicated that gR2-driven FL-IFN-γR2/GFP expression, though detectable, was very low compared to CMV or EF-driven FL-IFN-γR2/GFP expression. gR2-driven IFN-γR2 was able to reconstitute signaling in hamster cells stably expressing IFN-γR1, however, the MHC Class I surface antigen induction and Stat1 activation rate were lower than that seen in cells reconstituted with CMV or EF-driven IFN-γR2. Though anti-VSV activity was never observed in hamster cells, anti-EMCV activity in hamster cells was observed in response to both hamster and human IFN-γ, but **only** in cells expressing gR2-driven IFN-γR2. The results of this report indicate that pathways conferring protection against EMCV and VSV resistance differ as was noted by others and that low levels of IFN-γR2 expression are necessary to observe anti-EMCV protection in hamster cells in response to IFN-γ. This latter result extends observations in which alteration of the levels of IFN-γR1 and IFN-γR2 modified a subset of IFN-γ bioactivities.

22019

ACTIVATION OF NF-κB BY PKR THROUGH ITS INTERACTION WITH THE IκB KINASE COMPLEX CATALYTIC SUBUNIT IKKβ.

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The interferon (IFN) induced dsRNA activated protein kinase PKR mediates inhibition of protein synthesis, through phosphorylation of the translation initiation factor eIF2α. It is also involved in gene induction through activation of the transcription factor NF-κB. In resting cells, NF-κB is retained in the cytoplasm through binding to inhibitors of the IκB family. It is released after phosphorylation of IκB by the IκB kinase complex (IKK), allowing its translocation to the nucleus. We have recently shown that PKR activates NF-κB, independently of its kinase function, by activating the IKK complex. Indeed, PKR interacts with the IKK complex through its major catalytic subunit, IKKβ. This interaction does not require the integrity of the IKK complex, as it occurs in cells deficient for NEMO, a structural and regulatory subunit of the IKK complex. We have performed a yeast two-hybrid screen of a human cDNA liver library in order to search for PKR partners. We have identified a few candidates, interacting strongly with PKR, among which the TAR RNA binding protein, TRBP. This cellular protein has already been reported to both heterodimerize with PKR and inhibit its ability to block protein synthesis (*Benkirane et al,1997,EMBO J, 16*). We are currently analysing the effect of TRBP and of the other PKR partners that we have identified on the interaction of PKR with the NF-κB signaling pathway.

22018

DIABETIC HEMODIALYSIS PATIENTS TEND TO HAVE HIGHER INTERLEUKIN 6 LEVELS THAN THE HYPERTENSIVE PATIENTS

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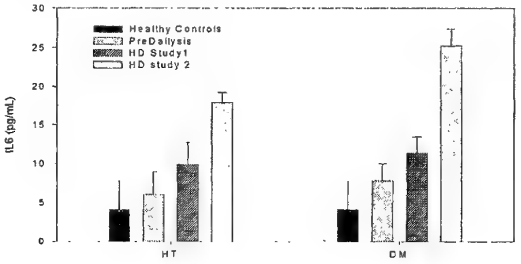
Interleukin 6 (IL-6) is a proinflammatory cytokine which is strongly correlated with nutritional status and patient survival. We studied the IL-6 levels in patients with diabetes (DM) and hypertension (HT) before (pre-dialysis group, N=42) and after the initiation of hemodialysis (HD study 2, N=234). Hemodialysis (HD) patients with no recent acute infections or hospitalizations were part of the HD study 1, N=90. Results are shown below (figure 1). Both DM and HT groups undergoing HD have elevated IL-6 values when compared with diagnosis-matched patients with advanced chronic renal failure. The diabetic hemodialysis patients have statistically significantly higher IL-6 levels than the HT group (HD study 1 and 2). These findings may provide an explanation for their poor prognosis while on hemodialysis. Efforts to identify the cause and prevent excessive cytokine production will help improve survival.



22017

IL-6 AUTOREGULATION DURING OSTEOCLASTOGENESIS AND BONE RESORPTION IN MOUSE TISSUE CULTURES AND IN MC3T3 OSTEOBLASTIC CELLS.

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IL-6 is thought to be an important mediator of osteoclastogenesis and bone resorption, particularly post-menopause and during increased PTH production. It has been suggested that IL-6 is capable of regulating the transcription of its own gene in certain cell types, including osteoblasts. The IL-6 receptor alpha chain is poorly expressed on osteoblasts and IL-6 often requires the presence of the soluble alpha chain (sIL-6R) for activity. The sIL-6R is still capable of combining with both ligand and the beta receptor chain (gp130) and inducing signal transduction. There is a lack of cross-reactivity between mouse (m) and human (h) IL-6 at the level of its interaction with the alpha chain, although all interactions between the ligand or the alpha chain and gp130 are species independent.The combination of hIL-6 and the hsIL-6R is capable of inducing strong responses in mouse cells. The use of an ELISA kit for mIL-6 which does not detect hIL-6, allows the study of the effects of IL-6 receptor stimulation on endogenous mIL-6 production in mouse tissues. The hIL-6-hsIL-6R combination (10-50 ng/ml - 50-200 ng/ml) induced osteoclastogenesis in co-cultures of bone marrow cells and primary osteoblasts and bone resorption in isolated neonatal calvaria. In parallel with these responses there was an increase in endogenous IL-6 production. This positive autoregulatory response is probably mediated via osteoblasts, as it was also observed in MC3T3 osteoblastic cells. Depending on the culture conditions, MC3T3 cells may respond to hIL-6 alone, presumably expressing sufficient alpha chain, or require the presence of the sIL-6R. In either case, stimulation of signalling via the IL-6 receptor complex was associated with an increase in endogenous mIL-6 release. All these effects were depended on both the concentrations of IL-6 and sIL-6R in the combination. This positive autoregulation of IL-6 may not be entirely direct, as a major component was blocked by inhibition of prostaglandin secretion with indomethacin (1μM), in all three test systems. The results in MC3T3 cells further emphasise the important autocrine role of prostaglandins in osteoblastic cells.

22016

TYPE I IFN INDUCES A UNIQUE TYPE OF PARTIALLY MATURE HUMAN DENDRITIC CELLS EXHIBITING UP-REGULATION OF CHEMOKINE RECEPTORS AND HIGH FUNCTIONAL ACTIVITIES.

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We recently reported that addition of type I IFN to GM-CSF-treated human monocytes resulted in the generation of partially mature dendritic cells (IFN-DCs) (J. Exp. Med., 191:1777-1788, 2000). We have now characterized the phenotypic and functional features of IFN-DCs as compared to the immature DCs obtained after GM-CSF/IL-4 stimulation (IL-4-DCs). IFN-DCs rapidly acquired the morphologic and functional features of DCs within 3 days. Of interest, IFN-DCs retained a phagocytic activity, even though expression of markers typical of mature DCs (CD83, CD25, TRAIL) was observed. Of interest, IFN-DCs exhibited high expression levels of both the chemokine receptors CCR5 and CCR7, whose expression is preferentially observed in immature and mature DCs, respectively. Likewise, IFN-DCs expressed higher amounts of MIP-3β and showed an enhanced capability to migrate in response to RANTES, MIP-1α and MIP-1β than IL-4-DCs. These results indicate that a unique type of partially mature DCs endowed with powerful functional activities is generated after a short-term exposure of GM-CSF-treated monocytes to type I IFN. We conclude that: i) type I IFNs can represent powerful vaccine adjuvants: ii) DCs generated in the presence of type I IFN may have advantages for DC-mediated immunotherapy strategies.

22027

Cloning and identification of SCL and its role in normal and pathological tissue growth

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Sarcolectin (SCL) is a potent stimulator of cellular DNA synthesis and an inhibitor of the Interferon (IFN)-dependent cellular functions. The cloned protein consists of 469 *aas* with four α helixes and two very short β sheets on each terminus which carry the lectin functions with a great number of potentially phosphorylated *aas* on the amino-end (19%) and on the carboxyl-end (36%); but in the intermediate helical domain only 9% are found. Antibodies raised to 15 synthetic *aas* on each terminus and in the transition domain of the α helix facilitate greatly the identification of the protein. SCL plays a pivotal role in T cell clonal expansion as well as in the normal growth of bones in co-operation with specific growth hormones. SCL is also likely involved in the induction of feed-back IFN responses necessary to arrest growth for differentiation. During several diseases such as AIDS or juvenile osteosarcomas SCL is overproduced, appears in the serum and can thus contribute to exacerbating the pathological manifestations. In such cases inhibiting SCL production or action might re-equilibrate the IFN-SCL balance and improve the clinical efficiency of the treatment.

22025

CO-OPERATIVE EFFECTS OF IL-11 AND PROSTAGLANDINS IN THE INDUCTION OF OSTEOCLASTOGENESIS AND BONE RESORPTION

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Some IL-6 family cytokines (IL-6 in the presence of its soluble receptor (sIL-6R), IL-11 and weakly OSM) induce osteoclastogenesis in co-cultures of mouse bone marrow cells and primary osteoblasts, and bone resorption in neonatal mouse calvaria. LIF and CNTF were ineffective. The effects of IL-6/sIL-6R, IL-11 and OSM involve prostaglandins as there were inhibited in the presence of indomethacin. IL-11 has been proposed as a central mediator in the increased osteoclastogenesis and bone resorption induced by several mediators, including PTH and 1,25 dihydroxy vitaminD3 (dhvitD3). hIL-11 (2-200 ng/ml) induced concentration-related increases in osteoclastogenesis and resorption with a maximal plateau effect from 50 ng/ml, associated with an increase in IL-6 production. In co-cultures, indomethacin (10^{-8}-10^{-6} M) induced a concentration-related inhibition of PGE2 release, and parallel reductions in osteoclastogenesis and IL-6 release induced by IL-11, dhvitD3 or 1-34 hPTH, despite a lack of increase in PGE2 production by PTH. In the bone resorption assay IL-11 or IL-1beta were prostaglandin-dependent but dhvitD3 and PTH were not. A neutralising anti-IL-11 monoclonal antibody (IL-11 Ab) blocked all the effects induced by IL-11 in these models. In co-cultures, the IL-11 Ab also markedly reduced osteoclastogenesis and IL-6 release induced by dhvitD3, PTH or PGE2. In calvaria, IL1beta responses were partially reduced but those to dhvitD3 and PTH were unaffected. Thus, the IL-11 Ab only blocks other mediators when their responses depend on prostaglandins. In the co-culture assay where there is relatively high basal release of PGE2, there may be reciprocal control between IL-11 and prostaglandins, with whichever is at higher levels stimulating the release of the other. The equilibrium so maintained would determine response levels, possibly via a synergy between the two mediators. This mechanism may also underly the responses to dhvitD3 and PTH in this preparation. However, in the calvaria preparation where there is relatively low basal production of PGE2, although the responses to IL-11 depend on prostaglandins, dhvitD3 or PTH do not depend on either prostaglandins or IL-11.

22024

NEW HUMAN AMNIOTIC CELL LINES USEFUL FOR SENSITIVE ANTIVIRAL AND ANTIPROLIFERATIVE ASSAY OF HUMAN AND PORCINE INTERFERONS

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Interferons (IFNs) are the molecules showing the antiviral, antiproliferative and immunomodulatory activity. To determine the antiviral (AV) activity mostly WISH and MDBK cells with VSV (Vsicular stomatitis virus) as challenge are used. In standard AV assay 500 I.U. of HuIFN-α is used as standard. Cells are cultivated in Eagle's medium + 10% of FCS (Fetal calf serum). In standard AV assay the MDBK cells are up to 5 times more sensitive for Human or Porcine IFN-α. On contrary for the antiproliferative (AP) activity there is not avaliable the standard protocol as well as proper cell line. Mostly, for AP activity the HEP-2 cell lines is used. The presented experiments were aimed to isolate a new human amniotic cell line and to test their's sensitivity for the AV and AP activity of Human and Porcine IFNs in comparison with standard cell lines. After amniocynthesis the cells were cultivated and characterised by chromosomal picture. Afterward further passages were made by using Eagle medium + 15% of Sheep's plasma. After the selection, new colonies of "transformants" appeared, and further on epithelial cells were obtained. Cells were designated as HAC-3/T2 and HAC-3/T3 (Human amniotic cells). They were than cultivated in Eagle's medium + 10% of SR-2 (Serum replacment), and for the AV test 2% only was used. The following IFNs were tested: HuIFN-α and γ and PoIFN-α and γ. In the AV assay in case of Hu or Po IFN-α the 5-10 I.U./ml could be determined. In case of Hu or PoIFN-γ 10-15 I.U in comparison to the NO assay. It can be concluded, that the new human amniotic (HAC-3/T2 and HAC-3/T3) represent a useful tool for the IFN analysis in the sera of the patients in case of Human and/or animals in case of Porcine Interferons.

22023

Effects of TNF on Fas and Fas-L expression by human endothelial cells from different body districts.

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One of the functions of endothelial cells (EC) coating the luminal surface of the vessel wall, is the control and regulation of inflammation in the tissues This function is driven by differential expression of adhesion molecules and may be regulated by cytokines. Moreover, EC have been shown to exert a cytotoxic activity towards Fas+ infiltrating lymphocytes due to the constitutive expression of Fas-ligand. On the other hand, EC themselves express Fas, although they are normally resistant to Fas-ligand induced apoptosis

Since apoptosis of EC may also be a crucial event in the development of inflammation, in the present study the effect of TNF-alfa stimulation on Fas and Fas-ligand expression on human EC has been investigated. EC from different districts (adult umbilical vein, aorta, heart and brain microvessels and embrional heart microvessels) were cultured in vitro by st andard techniques. At confluence, cells were either stimulated with TNF alfa (50 ng/mL) or not for 24 hours. Cells were than recovered and stained with anti CD31, anti Fas and anti Fas-ligand and analysed using flow cytometry

Constitutive expression of Fas and Fas-ligand was detected in all EC analysed with no differences among the various districts. A significant increase in the expression of Fas was detected after TNF alfa stimulation while – in our experimenthal conditions - no regulation was detected in Fas-ligand expression. To asses whether the TNF-induced increase in Fas expression was associated to an increased susceptibility to Fas-dependent apoptosis, EC were cultured in the presence of an anti-Fas antibody able to induce apoptosis. Preliminary results on embrional heart EC, showed that treatment with TNF was not able to increase the susceptibility of these cells to Fas-dependent apoptosis

22022

MODULATION BY IL-12 OF IL-8 PRODUCTION BY HUMAN NEUTROPHILS

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Interleukin-12 (IL-12) is a cytokine produced by phagocytes, mainly acting on T helper 1 and NK cells. However little is known on the pro-inflammatory effects of IL-12 on polymorphonuclear neutrophils (PMN). We thus tested the ability of IL-12 to modulate the production of the chimiokine IL-8 by PMN. **Methods**: Blood PMN from healthy volunteers were purified and cultured ($2\ 10^6$ /ml, up to 24h) with or without LPS and increasing doses of IL-12. Other stimuli such as IFNγ, TNFα and GM-CSF were tested as well as two inhibitors (IL-10 and dexamethasone). IL-8 was determined in the cell culture supernantants and lysates by ELISA (R&D) together with mRNA analysis. **Results :** IL-12 alone had no effect on PMN IL-8 production. By contrast, IL-12 had a synergistic dose- and time-dependent effect with LPS at both protein and mRNA levels, but in a lesser extent than IFNγ, TNFα or GM-CSF. IL-10 and dexamethasone were able to inhibit this effect of IL-12. Similar data were observed with both released and cell associated IL-8. The mechanism of this synergy seems to be mediated by TNFα and IFNγ. In conclusion, these results suggest a new role for IL-12 in the proinflammatory response by enhancing IL-8 production by PMN and thus contributing to PMN recruitment at the inflammatory sites by an autocrine regulation loop.

22015

STRUCTURAL CHARACTERIZATION OF *tsg-5*: A NEW TNF-RESPONSIVE GENE

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Tumor necrosis factor (TNF), a potent pro-inflammatory cytokine produced primarily by activated macrophages, elicits a large number of biological effects. To better understand the actions of TNF, we applied the Differential Display RT-PCR to compare the mRNA population of untreated and TNF-treated mouse embryonic fibroblasts (MEFs). Among several differentially represented bands, we cloned a 253 bp fragment of cDNA that was more prominent in lanes corresponding to TNF-treated cells, and that hybridizes with a mRNA of approximately 570 bases. Analysis of this cDNA sequence didn't reveal significant homology to sequences deposited in GenBank. This new gene was named TNF-stimulated gene 5 (*tsg-5*). By screening a cDNA library and a mouse genomic library, we characterized the complete *tsg-5* cDNA sequence as well as the *tsg-5* gene. The *tsg-5* gene consists of 3 exons with a putative initiation codon (ATG) located within the second exon and with an ORF of 120 nucleotides. We identified one specie of cDNA with an alternative splicing event in which exon two was skipped. This shorter mRNA has an ORF of 102 nucleotides that initiates in an alternative ATG codon within exon 1. In MEFs, *tsg-5* mRNA is augmented in response to TNF but not to IL-1 or IL-6. RT-PCR-based analysis of *tsg-5* gene expression *in vivo* revealed a constitutive expression of both mRNA species in thymus and ovary. However, in mice treated with LPS, we detected an induced expression of the larger transcript in spleen, brain and lungs with no detectable levels of the mRNA lacking the second exon.

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22014

IFN-α MEDIATED APOPTOSIS IS INDUCED IN THE PRESENCE OF THE PROTEIN TYROSINE PHOSPHATASE INHIBITOR VANADATE AND IS JAK/STAT DEPENDENT

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IFNs modulate several cellular responses including antiviral, antigrowth and immune function. Type I IFN-dependent inhibition of cell growth may occur either in the absence or presence of apoptosis. The mechanisms by which cells determine whether or not to undergo apoptosis after exposure to IFN-α are not clear. This study shows that 2fTGH, Jurkat and Hela cells, which are growth inhibited by IFN-α undergo apoptosis when incubated with both IFN-α and low concentrations of the protein tyrosine phosphatase inhibitor vanadate. In contrast, the combination of TNF-α with vanadate did not trigger apoptosis in these cells. Priming of 2fTGH cells with higher doses of vanadate for 6 hrs was sufficient to allow IFN-α to induce apoptosis without continuous exposure to vanadate. Caspase-3 activity was detected only in cells exposed to IFN-α/vanadate but not to IFN-α or vanadate alone. Activation of the Jak/Stat pathway and expression of interferon inducible genes was not altered by incubation of cells with IFN-α/vanadate compared with IFN-α alone. 2fTGH variants that do not express Stat1, Stat2, Jak1 or Tyk2 or express kinase dead forms of Jak1 or Tyk2 did not undergo apoptosis in the presence of IFN-α/vanadate. Reconstitution of these mutant lines with the appropriate wild type protein restored IFN-α/vanadate mediated apoptosis. These studies suggest that in addition to IFN-α stimulation of Stat-dependent genes, another signaling cascade may be required for this cytokine to induce programmed cell death and inhibiting the activity of a tyrosine phosphatase may play an important role in activating this other pathway to promote apoptosis.

22028

THE ROLE OF IL-1β, IL-1ra AND SUPEROXIDE DISMUTASE (SOD) IN MODULATION OF HOST REACTION TO X-RAY IRRADIATION

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The aim of this work is to study host reactions to X-ray irradiation. For modulation of these reactions IL-1b, IL-1ra and hrCuZnSOD were used. Mouse BALB/c and (CBAxC57Bl)F1 lines received 5.5, 6.5 or 7.5 Gy X-ray irradiation in dose intensity 0.54 Gy/min. It has been shown that IL-1β injection 24 h before irradiation induces 70-90% mice survival in dose dependent manner. Per oral application of IL-1β has given the same results. SOD protected 90-100% of mice from X-ray irradiation if it was injected 15-20 min before and 15-20 min and 4 h after irradiation. IL-1β and SOD which was used jointly has not shown any agonistic effects in doses of irradiation mentioned above. IL-1β and IL-1ra had opposite effects on growth of CFU-C in irradiated mice. IL-1β was protective for colony growth but IL-1ra canceled this effect.

22037

ANTIVIRAL AND CYTOKINE-INDUCING PROPERTIES OF ORGANOSELENIUM COMPOUNDS, ANALOGUES OF EBSELEN

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Ebselen (2-phenylbenzisoselenazol-3(2H)-one) is an antiinflammatory experimental drug. Ebselen and 218 of its analogues were studied as cytokine inducers and antiviral agents. Among the compounds were bisaryldiselenides, N-substituted benzisoselenazol-3(2H)-ones and other nitrogen and selenium containing heterocycles. Interferon, tumour necrosis factor and interleukin 10 production was determined in human peripheral blood leukocyte cultures treated with organoselenium compounds. Some of the compounds were found to be strong cytokine inducers comparable with phytohemagglutinin or lipopolysaccharide. Antiviral effects were studied *in vitro* with the use of DNA (herpes simplex virus 1) and RNA (vesicular stomatitis virus and encephalomyocarditis virus) viruses. Ebselen and many of the compounds were shown to have anti-HSV-1 and anti-EMCV properties. However, only weak direct anti-VSV effect was observed. The organoselenium compounds were found to be able to modulate the multiplication of the viruses, including VSV, in human cells. The relationships between structures and activities were observed. However, no exact correlation between cytokine-inducing and antiviral activities was found. The compounds showing strong cytokine-inducing or antiviral properties and low cytotoxicity in human cells were chosen for further studies. This work was supported by the Polish State Committee for Scientific Research (grant no. T09A 09717).

22040

TRANSIENT ASSOCIATION OF NFκB p50 WITH THE C-REACTIVE PROTEIN GENE PROMOTER ENHANCES C/EBP-MEDIATED CRP TRANSCRIPTION

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Transcription of the CRP gene in Hep 3B cells is stimulated by IL-6 but not by IL-1 and is synergistically activated by their combination. Part of the IL-6 response is observed with a 125 bp fragment of the proximal promoter which binds STAT3 and C/EBP isoforms. In previous studies we showed that overexpression of NFκB subunit p50 stimulates CRP transcription while overexpression of p65 repressed expression even in the response to cytokines. P50 but not p65 is capable of binding to a nonconsensus κB site overlapping the C/EBP binding site. To explore the mechanism by which p50 has its effects, we employed EMSA studies with nuclear extracts prepared from Hep 3B cells and a 24 bp oligonucleotide derived from the CRP promoter which included the C/EBP and nonconsensus κB sites. Cytokines induced three retarded bands which could be supershifted with antisera against C/EBPβ or C/EBPδ. The intensities of these bands could be diminished by preincubation with an antisera against p50 or competition with a consensus κB-containing oligo. Neither of these treatments altered the mobilities of the C/EBPβ-containing complexes. Transactivation experiments showed that the C/EBP binding site was essential for cytokine responsiveness and that mutation in the p50 binding site resulted in an 80% reduction in activity. We conclude that p50 is required for C/EBPβ binding to the proximal CRP promoter but that it is not a stable component of the complexes formed on the promoter. We speculate that transient interaction of p50 with the CRP promoter enhances C/EBP binding. We further speculate that sequestration of p50 by overexpressed p65 can prevent C/EBP complex formation even under conditions of C/EBP activation by cytokines. (Supported by NIH grant AG02467)

22041

THE IMPROVEMENT OF HUMAN LEUKOCYTE INTERFERON (HuIFN-α) PRODUCTION BY ELIMINATING OF INHIBITOR FROM RESIDUAL PLASMA OF LEUKOCYTES

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Human leukocyte interferons (HuIFN-α) are pleiotropic molecules showing the antiviral, antiproliferative and immunomodulatory activity. They are synthesized and released from leukocytes after Sendai virus induction. IFN-α derived from leukocytes consists from multiple subtypes, each with subtle differences in structure and biologic activity. HuIFNα is being produced in a quantities mostly by standard Cantell' s protocol: two-step lysis of erithrocytes, 2-hour priming of remained leukocytes ($0.8x10^7$/ml) with 200 I.U. of HuIFN-α and induction by 150 HAU/ml of Sendai virus followed by 18 hour incubation at 37°C. Despite the constant use of this, an evident variability in IFN's titers could be seen. Antiviral activity was tested on the WISH cells with a VSV as a challenge. The range from: 21.6 - 190.3 x 10^3 I.U./ml was found. The only variables were the "buffy coat" bags. Due to this, the existence of IFN's inhibitor in some of them can be speculated. To eliminate the inhibitor 10 bags were taken. Half of each was carried out by standard procedure, a second half, was a first washed out in excesse of PBS (Phosphate buffer saline) and than being pooled. The concentration of leukocytes before priming was $0.1x10^7$/ml. To enhance the IFN yield the primer was added directly into the leukocyte pool (60.000 I.U/bag) before the further procedure. In the control experiments the normal 200 I.U./ml priming was excluded. The obtained data shows: (1) Washing of the leukocytes probably remove the inhibtor, and consequently 3-5 times higher titers were found; (2) Direct priming could additionally increase the titer. (3) Addition of the primer directly into the leukocyte pool could not replace the standard priming. Using all these improvements the production became more uniform, with higher titers in a range of 115.7 - 165.9 x 10^3/ml.

22042

CELL-SPECIFIC EFFECTS OF INTERLEUKIN-17 ON NITRIC OXIDE PRODUCTION

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IL-17 is a T cell-derived cytokine that may play an important role in the initiation or maintenance of the inflammatory response, enhancing the transcription of genes encoding proinflammatory molecules. Whereas expression of IL-17 is restricted to activated T cells, the IL-17 receptor is found to be widely expressed, a finding consistent with the pleiotropic activities of IL-17. In an effort to elucidate the biological role of IL-17 during inflammatory conditions, we investigated the effect of this cytokine to induce or modulate production of nitric oxide (NO) in rodent cells, having in mind important roles ascribed to NO in inflammatory diseases. We used murine or rat macrophages, primary astrocytes, as well as L929 fibroblasts. Neither of the examined adherent cells produced NO in the presence of IL-17 alone. However, IL-17 synergistically enhanced the induction of NO by IFN-γ, IL-1β or their combination in both astrocytes and fibroblasts. This production of NO was dependent on synthesis and activity of inducible NO synthase (iNOS), as indicated by elevated expression of iNOS mRNA (RT-PCR), and abrogation of nitrite accumulation with protein synthesis inhibitor, cycloheximide or selective inhibitor of iNOS, aminoguanidine. On the other hand, cytokine-stimulated NO production by macrophages was not influenced by the presence of IL-17, thus indicating involvement of distinct signaling pathways associated with iNOS induction in different cell types. Since it was recently hypothesized that contrasting, destructive and immunosuppressive effects of NO during inflammatory conditions, might be ascribed to macrophage or resident cell-derived NO, respectively, our results suggest important role for IL-17 in the control of the autoimmune and other inflammatory processes.

22043

SELECTIVE REGULATION OF ICAM-1 EXPRESSION BY IL-18 AND IL-12 ON HUMAN MONOCYTES

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IL-18 is a cytokine that plays an important role in the Th1-type response, primarily by its ability to induce IFN-γ production in a synergistic way with IL-12 by T cells and natural killer cells. In addition, IL-18 is able to induce synthesis of TNF and IL-1, two cytokines known to stimulate expression of adhesion molecules. The aim of this study was to assess the effect of IL-18 and IL-12 on adhesion molecule expression on human PBMC. The following molecules were investigated: LFA-1, Mac-1, P150, β_2, VLA-4, VLA-5, β_1, ICAM-1, ICAM-2, ICAM-3 and ALCAM. Furthermore, the role of endogenous IL-18 and TNF was studied in LPS-induced adhesion molecule expression by using IL-18 binding protein (IL-18BP), a natural inhibitor of IL-18, and TNF-BP. PBMC were isolated from human blood, incubated for 24 hours with either IL-18 (1μg/ml) and/or IL-12 (10ng/ml), LPS (1μg/ml) and analysed on a FACS for expression of the adhesion molecules listed. Stimulation of PBMC with IL-18, IL-12 and IL-12+IL-18 induced a 90%, 136%, and 270% increase in expression of ICAM-1 (CD54) on monocytes respectively ($P<0.05$). The effect of IL-18 and IL-12+IL-18 was dependent on endogenous TNF, since TNF-binding protein (TNF-BP) (10μg/ml) inhibited the enhancement of ICAM-1 expression with 28% and 37% respectively ($P<0.05$). Neutralizing endogenous IL-18 or TNF by IL-18BP (500ng/ml) or TNF-BP (10μg/ml) slightly, but significantly decreased LPS-induced ICAM-1 expression (14% and 17% respectively, $P<0.05$). In conclusion, IL-18 and IL-12 selectively upregulate ICAM-1 expression in monocytes, through a TNF-dependent mechanism and this pathway also plays a role in ICAM-1 induction by bacterial stimuli such as LPS.

22044

INTERFERON GAMMA IN PREIMPLANTATION MOUSE EMBRYOS : NEW FINDINGS AND HOPES.

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Interferon gamma (IFNγ) induces a cellular response in cells bearing specific two-chains receptors. This response occurs *via* a complex signaling pathway, involving JAK kinases and STAT transcription factors. In the frame of a more general search for possible intranuclear accumulation of interferon gamma [1], we have investigated the possible presence of IFNγ receptors (IFNγR) in mouse preimplantation embryos. We have detected by indirect immunofluorescence both receptor chains (IFNγRα and IFNγRβ) at the surface of mouse oocyte (in germinal vesicle and metaphase II stages), as well as at all stages of *in vitro* developping embryos from 1 cell to blastocysts. This was confirmed by RT-PCR experiments. In addition, IFNγ was found to bind to the oocyte and embryo membrane where it colocalizes with the receptor. More intriguing, we detected by indirect immunofluorescence the presence of intracellular IFNγ in embryos not previously exposed to the cytokine, suggesting its endogeneous synthesis by the embryos themselves. Indeed, the presence of interferon gamma mRNA was detected by RT-PCR at all embryonic stages analyzed. These findings open new ways for research on possible role of interferon gamma on preimplantation development of mammalian embryos, as well as on their capacity to secrete this cytokine [2].

1 Bader T and Wietzerbin J (1994). Proc Natl Acad Sci USA, 91 : 11831-11835.

2 Hill JA (1992). Am J Reprod Immunol, 28 : 123-126.

22045

CONTROL OF GROWTH AND EXPRESSION OF CELL REGULATORY GENES BY ONCONASE AND INTERFERON-BETA (IFN_B) IN ANDROGEN-REFRACTORY JCA-1 HUMAN PROSTATE CANCER CELLS.

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To explore the potential of using IFN and onconase in treating metastatic prostate cancer, we studied modulation of growth control and specific gene expression in hormone-independent JCA-1 cells by these two antitumor agents. Cells were treated with 1 and 5ug/ml of onconase, and additionally with 1000 IU/ml recombinant IFN-B_{ser}, for up to 3 days. Cell count, and incorporation of radioactive precursors into protein and RNA, were determined. Total expression and sub-cellular distribution of STAT1, p53, and NFkB were measured by Western blot analysis. Cells treated for 3 days with IFN or onconcase showed a 15 to 30% reduction in proliferation, which was increased to 42-51% when both agents were administered together. Synergism between these agents was confirmed by labeling experiments with [^{35}S] methionine; IFN and 1ug/ml onconase each produced a 23-25% decrease, while the combination gave a 59% suppression, of protein synthesis. Similar though smaller changes were also observed in RNA synthesis, measured as reduction in [^{3}H]uridine incorporation. Turnover of pre-labeled cellular RNA and proteins was not affected by either agent or their combination. Expression of STAT1 was increased 3-fold by IFN, and was reduced by 20-25% with addition of onconase. Phosphorylated and unphosphorylated forms of STAT1 were similarly affected by onconase. p53 level was significantly reduced by onconase. Expression of NFkB was increased 30 and 67%, respectively, by treatment with 5ug/ml onconase and IFN. Since changes in these three proteins were not correlated with the reduction in cell growth and the overall suppression of de novo protein synthesis, it may be suggested that there are other targets primarily responsible for the combined effect of IFN and onconase in prostate cancer cells. (Supported by an unrestricted grant from the Philip Morris Co. to Dr. J.M. Wu)

22046

IL-4δ2 COULD FUNCTION BOTH AS ANTAGONIST AND AGONIST OF IL-4

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We had studied effect of recombinant human IL-4 (rHuIL-4) and splice-variant IL-4δ2 (rHuIL-4δ2) on epithelial and lymphoid cells of human thymus. It was shown, that rHuIL-4 stimulated mitogen-induced thymocyte proliferation. rHuIL-4δ2 acted as its antagonist and caused inhibition of thymic lymphoid cell proliferation. At the same time, rHuIL-4δ2 potentiated proliferation of thymocytes stimulated by IL-2, increasing inclusion of the labeled thymidine by 45-50%. rHuIL-4 in dose of 1÷10 ρg/ml stimulated intact thymic epithelial cells (TEC), and in dose of 10÷100 ρg/ml inhibited their proliferative reaction. rHuIL-4δ2 addition in the test-system did not change the reaction of epithelial cells. rHuIL-4 stimulated proliferative response of TEC preliminary incubated with mitogen, the effect being intensified in the presence of IL-4δ2. TEC were determined to express on their surfaces high affinity receptors, being mutual both for IL-4 and IL-4δ2 ($K_d = 10^{-10}$ M).

22047

Expression of the IFN-induced GTPase, mGBP-2, alters cell adhesion

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The guanylate binding proteins (GBPs) are a family of unusual GTPases that are robustly induced by IFNs. Despite their abundance in IFN-exposed cells, little is known about their function(s). To study the function of murine mGBP-2, we generated NIH 3T3 cells stably expressing mGBP-2. Constitutive expression of mGBP-2 in NIH 3T3 cells altered their actin cytoskeleton, resulting in greater membrane ruffling and lamellapodia formation. In addition, these cells adhered more slowly when plated on tissue culture dishes. That this reflects a differential ability to recognize extracellular matrix proteins was verified by demonstrating that mGBP-2 expressing cells showed reduced adhesion to fibronectin, compared to control transfectants. No consistent difference in the ability of these cells to adhere to laminin was observed, suggesting that these differences do not reflect global changes in adhesion. IFN-γ treatment of human monocytes or mast cells has previously been demonstrated to result in reduced adhesion to fibronectin. We propose that mGBP-2 is an important component in the mechanism by which IFN alters cell adhesion, a process with ramifications for metastasis and immune recruitment. The mechanism by which mGBP-2 mediates these changes is currently under active investigation.

22048

INTERLEUKIN 16 STIMULATES HUMAN MONOCYTES TO PRODUCE IL-10 AND IL-18: EFFECTS ON IFNγ PRODUCTION

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In previous studies we have shown that IL-16 has an indirect effect on IL-12 driven IFNγ production. Whereas stimulation with IL-16 alone does not result in IFNγ production, the co-stimulation with IL-12 and IL-16 together increases the amount of IFNγ secreted compared to that produced in response to IL-12 alone. In this study we show that $CD14^+$ monocytes and total PBMCs stimulated with IL-16 produce IL-10 and IL-18. Our proposed model suggests that IL-16 stimulates monocytes to produce IL-18 which acts *in* synergy with IL-12 on natural killer cells to produce IFNγ. After depletion of the monocytes, the resting cells produce more IFNγ than total PBMCs suggesting that monocytes in PBMCs have an inhibitory effect on IFNγ production. This effect is probably due to the production of IL-10 by monocytes. IL-10 production by unstimulated PBMCs and isolated $CD14^+$ monocytes could be readily detected, the levels of which were enhanced by addition of IL-16. IL-10 is an anti-proliferative cytokine and the effect of IL-16 driven IL-10 production is being examined using assays measuring ^{3}H-thymidine or BrdU incorporation. In addition the quantification of IL-10 RNA is currently being performed.

22038

ANTI-IL-9 AUTOANTIBODY INDUCTION : A TOOL FOR THE STUDY OF CYTOKINE FUNCTION IN VIVO

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Neutralizing anti-IL-9 auto-antibodies were induced in mice by immunization with mouse IL-9 coupled to ovalbumin. This response was long-lasting and abolished IL-9-induced mast cell activation and hypereosinophilia.
We took advantage of this model to assess the role of IL-9 during infections by *Trichuris muris*, as IL-9 production has been reported to correlate with resistance against this nematode. C57BL/6 mice became susceptible to the infection when they were subjected to anti-IL-9 immunization, demonstrating that IL-9 plays a critical role in this model.
The anti-IL-9 antibody response was further analyzed by generating monoclonal anti-IL-9 antibodies from IL-9-OVA-vaccinated mice. Analysis of these antibodies proved that, in spite of their auto-immune nature, sub-class switching and affinity maturation occurred normally.

22021

iPFK-2 Mediates the Metabolic Activation of Macrophages and T cells: Control Point for ATP and Nucleotide Precursor Production

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We recently reported the cloning of an inducible isoform of 6-phosphofructo-2-kinase (iPFK-2) that bears a cytokine-like, AU-rich instability element in its 3'-UTR (*PNAS*, 96:3047-3052, 1999). Among known PFK-2 isoforms, iPFK-2 also is unique in that its promoter contains 5 potential binding sites for NF-κB. iPFK-2 functions to produce fructose 2,6-bisphosphate (F2,6BP), a powerful allosteric activator of PFK-1, the rate limiting step of glycolysis. Like other AU-rich containing genes, iPFK-2 mRNA and protein expression increases in monocytes/macrophages upon pro-inflammatory activation by LPS. Moreover, antisense blockade of iPFK-2 expression decreases intracellular F2,6BP levels and glycolytic flux, as well as synthesis of 5-phosphoribosyl-1-pyrophosphate, the product of the pentose phosphate shunt that is a critical precursor for *de novo* RNA and DNA synthesis. T cell expansion by foreign antigen also is known to be dependent upon increased glycolysis, which supports the ATP and anabolic requirements of intracellular signaling pathways, membrane turnover, and protein and nucleic acid synthesis. We postulate that the normal T cell activation response requires iPFK-2 expression to effect antigen-stimulated glycolysis. We purified human CD4+ T cells, stimulated them with anti-CD3 and anti-CD28 mAbs, and analyzed iPFK-2 mRNA expression by RT-PCR and Western blotting. iPFK-2 mRNA and protein levels increased significantly after 24 hours and were sustained for at least 72 hours of culture. The induction of iPFK-2 coincided with an increase in F2,6BP production (control, 12.3±3.5 pmol/mg protein; +αCD3/αCD28, 28.6±5.3 pmol/mg protein; p=0.011), lactic acid secretion (control, 0 094±0.008 mg/ml; +αCD3/αCD28, 0.72±0.04 mg/ml; p=0.007), and cell proliferation (control, 1823±79cpm; +αCD3/αCD28, 13434±295cpm; p<0.001). Anti-IL-2 (50 µg/ml) or dexamethasone (10^{-8} M) significantly attenuated iPFK-2 expression in activated T cells. iPFK-2-catalyzed production of F2,6BP may be an essential component of the metabolic response to immune cell activation and may represent an important target for the development of novel immunoregulatory agents.

22036

DOMAIN STRUCTURE OF PACT, THE PROTEIN ACTIVATOR OF PKR

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PKR, a latent protein kinase, mediates transcriptional signaling by many cytokines and growth factors. It is also a major regulator of translation. We have previously identified a human protein, PACT, that can directly bind to PKR and activate it by promoting its autophosphorylation. Here, we have analyzed the domain structure of PACT. For these studies, we used *in vitro* and *in vivo* PKR-binding and activation assays. PKR activation *in vitro* was measured by its PACT-induced autophosphorylation and PKR activation *in vivo* was measured by translational inhibition and cellular apoptosis. Our investigation identified three distinct domains of PACT. Domains 1 or 2 mediated high affinity binding of the protein to PKR and the presence of either of these domains was sufficient for PACT functions. Domain 3, on the other hand, was indispensable for PKR activation and it could, by itself, efficiently activate PKR *in vitro*. *In vivo*, it failed to do so because of its poor affinity for PKR at a physiological salt concentration. When strong binding was restored by attaching a heterologous PKR-interacting domain, domain 3 could activate PKR *in vivo* as well and cause apoptosis. These studies demonstrated that PACT has a modular structure with two PKR-binding domains and one PKR-activation domain.

22020

REGULATION OF EXPRESSION OF THE PANCREATITIS ASSOCIATED PROTEIN FAMILY BY LEPTIN AND IL-6

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The pancreatitis associated protein family is composed of secreted lectin-related proteins, differentially expressed in a tissue-specific manner and participating in pancreas in acute phase response. Previously, RDA (representational difference analysis) allowed us to clone amplicons for PAP family members from leptin and IL-6 stimulated PC12 cells. Here, differential expression of the PAP family was studied in non-differentiated PC12 cells. Rat PAP I was weakly induced by leptin but forskolin had a strong co-stimulatory effect. Stronger induction was obtained via gp130 (by IL-6α) in which case forskolin showed a down-modulation. Similar data were obtained for rPAP II, rPAP III and REG I. Dexamethasone revealed a slightly inhibitory effect on rPAP I induction by leptin. In differentiated PC12 cells, leptin, leptin/dexamethasone, nor dexamethasone alone could significantly induce PAP II, PAP III or REG I. A functional study of the rPAP I promoter was performed to explore regulatory domains involved in leptin-dependent induction. Progressive rPAP I promoter deletions were analysed in transiently transfected PC12 cells stably expressing the leptin receptor long form. The effect of leptin, forskolin, dexamethasone, β-NGF, IL-6 + sIL-6Rα, or combinations thereof was studied. Our data are consistent with the pattern of consensus regulatory sequences in the rPAP I promoter but at the same time argue for the presence of new regulatory sites. Furthermore, forskolin and β-NGF may interfere at higher level in the signalling cascade.

22035

RECOMBINANT INTERFERON-α2: ITS PSYCHTROPIC AND ANTI-STRESS EFFECT

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According to our data, endogenic pyrimidines, such as uridine, thymidine, orotate and etc. possessed universal effect on the regulation of neuropsychic and immunological responses. It was very important to study the role and place of the cytokines, especially of the interferon system. It was shown, that manifested distinct stimulating interferon effect on their spontaneous motor activity only in an hour after interferon injection, especially in dose of 2500 IU/kg. Those effects had been preserved till the third hour of observation. Different doses of interferon, apparently, switched different mechanisms or levels of locomotor activity regulation. Stress-protective effect of interferon had been studied on the models of emotionally-painful and heat influences. Interferon decreased the frequency and lowered the size of hemorrhages on stomach mucus developed after electro-pain stress irrespective of the dose and prevented spleen weight reducing. Heat stress was reproduced in thermostat with air temperature of 39°C and relative humidity of 75-80% for 25 min. during 3 days. Spleen weighting after 3 day heat load demonstrated, that in control spleen weight was 67.8±2.4 mg, and on the interferon background in the dose of 2500 IU/kg, it was 93.4±2.9 mg. The preparation did not produce statistically important influence on thymus weight. After interferon injection in dose of 125 IU/kg, large (more than 1.5 mm in diameter) subserous hemorrhages on the stomach walls were absent. Interferon, especially in the dose of 2500 IU/kg, essentially increased provoked with electro-painful stimulation agression of white non-breed mice and significantly lowered the pain appreciation (algesthesia). Found neutopsychotropic interferon effects demonstrated its ability to increase animal agression and to have psychostimulating, antinociceptive and antistress action.

22034

THE CYTOKINE mRNAs IN CELL LINES WITH DIFFERENT IFN-SENSITIVITY

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Nowadays the role of cell IFN-sensitivity is taken into view while analyzing many pathological conditions following cellular homeostasis failure in infectious, oncological or immunological diseases. However little is still known about the interrelationship of cell IFN-sensitivity and cytokine production. Continuous cell lines J-96 and J-96-derived J-41 subline, differing by sensitivity to antiviral and antiproliferative action of IFN-α were used to study mRNA synthesis for cytokines IL1β, IL2, IL4, IL6, IL10, TNF-α, IFN-α and IFN-γ. Cells were treated with IFN-α (1000 IU/ml) or inhibitor of IFN action (IIA, 16-32 IU/ml). Total RNA isolated from the cells using Rneasy Total RNA System (Qiagen, Santa Clarita, CA) and cDNA was synthesized from 2 mg of total RNA using oligo dT primers and AMV reverse transcriptase (both from NewEngland Biolabs, Bedford, MA). The cDNA was used as a template in the RT-PCR reaction with the primer pairs. Cells of J-96 line susceptible to IFN-α were found to constitutively express mRNAs for cytokines IL1β, IL6, TNF-α; IFN-α; IFN-γ. In J-41 cells, non-sensitive to IFN-α, the same mRNAs were expressed constitutively, except mRNAs for IFN-α and IFN-γ. Following treatment with IFN-α, except constitutively presented cytokines mRNAs, in cells J-96 induction of IL2 and IL4 mRNAs was additionally found. Meanwhile, similar treatment of J-41 cells was accompanied by inhibition of earlier found constitutively expressed mRNAs for IL1β, IL6 and TNF-α. After treatment of J-96 cells with IIA no change in mRNA levels was seen compared with control culture. Both IIA and IFN inhibited constitutive mRNA synthesis in J-41 cells. Thus we shown here that interferons and inhibitors action upon the cells non-sensitive to IFN led to inhibition of the cytokine mRNAs. Meanwhile the action of IFN-α upon the cells of IFN-sensitive line resulted in the cytokine mRNA synthesis induction. Thus the results shown above might be the basis for recommendation to measure the IFN-sensibility when study clinical aspects of IFN and other cytokines usage.

22039

MIF MODULATES ADHESION MOLECULE EXPRESSION BY ENDOTHELIAL CELLS *IN VITRO*.

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Macrophage migration inhibitory factor (MIF) has been shown to play an important role in the regulation of host inflammatory responses. Previous studies have revealed that immunoneutralization of MIF inhibits the progression and severity of several experimental models of inflammatory diseases, including arthritis, endotoxemia and exotoxemia, as well as glomerulonephritis. More recent studies have shown that anti-MIF antibodies inhibit tumor-associated angiogenesis (*Mol. Med.* 5: 181, 1999; *Cytokine* 12: 309, 2000). These studies also demonstrated that MIF was mitogenic for endothelial cells *in vitro*. Endothelial cells are actively involved in inflammatory disease processes by expression of adhesion molecules, transmigration of inflammatory cells into subadjacent tissues, production of pro-inflammatory cytokines, and angiogenesis. We have investigated the potential role of MIF in endothelial cell activation by examining the effect of recombinant MIF on adhesion molecule expression *in vitro* using flow cytometry methods, as well as a cell-based ELISA system. Recombinant MIF (50-250ng/ml) treatment of microvascular endothelial cells upregulates E-selectin and ICAM-1 expression maximally at 6 and 28 hrs, respectively, in a dose-dependent manner. MIF treatment of microvascular endothelial cells also induces VCAM expression maximally at 18-28 hrs. The functional activities of the adhesion molecules were assessed using inflammatory cell adhesion assays with MIF treated endothelial cell cultures. In addition, we have also shown that rMIF promotes pro-inflammatory cytokine release by endothelial cells. The precise role of MIF during the inflammatory response has not yet been elucidated. These studies suggest the potential role of MIF in endothelial cell activation during inflammation.

Signal transduction II

13013

RPM, smgGDS and M-Ras: novel Ras pathways

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Growth factors acting on receptors of either the cytokine superfamily such as IL-3 or GM-CSF, or of the receptor–tyrosine-kinase family, such as SLF, have been proposed to activate the classical p21 Ras proteins H-Ras, N-Ras and K-Ras. However, the p21 Ras proteins share about 50% amino acid identity with a new member of this super-family, M-Ras. The principal tools used to assess the activation or functional significance of p21 Ras, the monoclonal antibody Y-13 259 and dominant-negative mutants of Ras, fail to discriminate between M-Ras and p21 Ras, raising uncertainty about which of Ras super-family members are involved in particular processes, such as signalling downstream of cytokine receptors. M-Ras is expressed ubiquitously and is acted upon by the same exchange factors and GAP's that are active on p21 Ras. Using mass-spectroscopy, we identified one of the proteins interacting with M-Ras as the atypical exchange factor, smgGDS. smgGDS interacted selectively with the GDP-bound form of M-Ras and co-expression of smgGDS and wild type M-Ras resulted in activation of M-Ras. Although M ras is oncogenic, it does not strongly activate known growth-promoting paths. Using yeast two-hybrid screens we identified a novel effector of M-Ras, RPM which was shared by and p21 Ras. This protein, RPM is a new member of the Ral GDS family of exchange factors and selectively bound GTP-loaded p21 Ras or M-Ras. Co-expression of either RPM or mutants of RPM lacking a Ras-binding domain, inhibited activation of an Elk reporter mediated by activated p21Ras or MEKK1, indicating that RPM has a novel inhibitory effect on signalling. M-Ras is highly conserved in evolution with an ortholog in *C. elegans*: and ongoing analyses of mice with disruption of the M Ras gene are likely to reveal distinctive functions.

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13011

ACTIVATION OF THE RAC1/P38 MAP KINASE PATHWAY BY TYPE I IFNS REGULATES TRANSCRIPTIONAL ACTIVATION VIA SERINE PHOSPHORYLATION OF HISTONE H3.

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We and others have recently demonstrated that the p38 Map kinase pathway is activated by IFNα and IFNβ and that its function is essential for gene transcription via ISRE elements. We now identify the small G-protein Rac1 as an upstream regulator of p38 activation by Type I IFNs. Our data demonstrate that IFNα and IFNβ induce rapid activation of Rac1 and that such an activation is tyrosine kinase-dependent. Overexpression of a dominant negative form of Rac1 blocks IFNα-dependent activation of p38, indicating that Rac1 is required for engagement of the p38 pathway. In other studies we establish that p38 activation is essential for Type I IFN-induced gene transcription via GAS elements and via the promoter of the PML gene, which mediates growth inhibitory responses. Inhibition of the p38 pathway abrogates transcriptional activation without decreasing the formation of various Stat- DNA binding complexes or phosphorylation of Stat1 or Stat3 on Ser727. To identify the mechanisms by which p38 mediates gene transcription by Type I IFNs, we determined whether IFNα induces nuclear histone phosphorylation via a p38 dependent mechanism. Our data demonstrate that IFNα induces phosphorylation of histone H3 at serine 10 and that such phosphorylation is inhibited by the p38 specific inhibitor SB203580. Thus, the major function of the Rac1/p38 pathway in IFN-signaling appears to be serine phosphorylation of nuclear H3, which regulates chromatin remodeling and immediate early gene expression.

13007

Function of IRAK in IL-1-signaling and identification of Act1, a novel NFκB-activating protein

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Mutagenized human 293 cells expressing thymidine kinase under control of the E-selectin promoter have yielded many mutant clones unresponsive to IL-1. A clone lacking IRAK (IL-1 receptor-associated kinase) (I1A) was used to show that the kinase activity of this protein is not required for signaling in response to IL-1. IL-1-induced phosphorylation, ubiquitination and degradation of IRAK correlate with its ability to restore the IL-1 responsiveness in I1A cells. N-terminal domain (residues 1-103) of IRAK is required for the IL-1-induced activation of both NFκB and Jun kinase (JNK), whereas the following region (residues 104-198) is only necessary for NFκB activation, suggesting that the IL-1-induced NFκB and JNK activation may diverge at the level of IRAK. The other isolated IL-1-unresponsive clones do not lack any of the known components in IL-1 signaling pathway and are being complemented by retroviral cDNA library. We recently cloned a novel NFκB activator (Act1). Act1 associates with and activates IκB kinase (IKK), leading to the liberation of NFκB from its complex with IκB. Many signaling pathways that liberate NFκB also activate ATF and AP1 through JNK. Act1 also activates JNK, suggesting that it might be part of a multifunctional complex involved in the activation of both NFκB and JNK. Act1 fails to activate NFκB in an IL-1-unresponsive mutant cell line in which all known signaling components are present, suggesting that it interacts with an unknown component in IL-1 signaling.

13005

JAK1 interacts with gp130 through its FERM domain

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The FERM (band Four-point-one, Ezrin, Radixin, Moesin) homology domain was first identified in the N-terminal region of cytoskeletal proteins and was shown to be involved in targeting these proteins to the plasma membrane. Recently, the presence of a FERM-like domain has been demonstrated in the N-termini of a wide variety of proteins, including the JAKs. The FERM domains of the JAKs are approximately 400 amino acids in length and contain 19 conserved hydrophobic regions. The objective of the current study was to determine the domain(s) of JAK1 required for recruitment to the gp130 subunit of the IL-6 receptor. To this end the interaction of a number of JAK1/JAK3 chimeras with: (i) gp130 and (ii) a biotinylated gp130 Box1/Box2 JAK-binding-domain peptide, was investigated. Chimeras I and II (JAK1 FERM domain conserved regions 1-19 and 1-18/JAK3, respectively) co-immune precipitated with gp130 although less efficiently than JAK1. JAK3 and Chimera III (JAK1 FERM conserved regions 1-8/JAK3) did not co-IP. Similar results were obtained in the biotinylated peptide pull-down assays. All three chimeric JAKs were catalytically active in an autophosphorylation assay. Chimera II restored gp130 signalling in JAK1-negative cells but poorly, possibly reflecting some degree of substrate specificity for the JAK1 versus the JAK3 kinase domains. Overall, however, the data are consistent with recruitment of JAK1 to gp130 (Box1/Box2) through the JAK1 FERM domain

13001

NEW TYK2 PARTNERS IDENTIFIED BY A YEAST TWO-HYBRID SCREEN

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Tyk2, a member of the JAK tyrosine kinase family (Tyk2, JAK1, JAK2, JAK3), is associated with several cytokine receptor, including the IFNα/β, IL-10 and IL-12 receptors. All JAK proteins possess a carboxy-terminal catalytically active tyrosine kinase domain [JH1], a regulatory kinase-like domain [JH2], and an amino-terminal region [JH3 to 7] containing the structural determinants of binding to cytokines receptors. The functional domain organization of the large amino-terminal moiety of the JAKs is far from being understood. Recently, sequence analysis of this region has revealed the presence of a duplicated band 4.1-related domain. This domain, also named JEF or FERM, is found in several proteins such as the ERM (Ezrin, Radixin, Moesin), FAK and PYK2, and is thought to mediate intramolecular interactions with transmembrane proteins. In order to identify potential new partners of Tyk2 and to further study the role of the amino-terminal region, we performed a yeast two-hybrid screen using, as bait, the first 450 aa (both JEF domains) of Tyk2. Eleven positive clones were isolated, six of them containing overlapping sequences. Sequence analysis has revealed that these cDNAs represent novel genes. *In vivo* interaction between Tyk2 and the encoded protein segments was confirmed for four independent clones by coimmunoprecipitation using transiently transfected Cos-7 cells. Experiments are in progress to characterize these proteins and to analyze the physiological role of their interaction with Tyk2.

13002

JAB1 IS A/THE BINDING PROTEIN FOR THE CYTOKINE MIF: MODULATION OF AP-1 ACTIVITY AND CELL CYCLE PATHWAYS

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Macrophage migration inhibitory factor (MIF) is one of the first cytokines discovered and exhibits typical properties of an inflammatory cytokine, but also displays unique endocrine and enzymatic functions. Anti-MIF-based strategies bear therapeutic potential. The molecular targets of MIF action have not been identified. Here we show that MIF specifically interacts with an intracellular protein, Jab1, a coactivator of AP-1 transcription that also promotes degradation of the cyclin-dependent-kinase inhibitor $p27^{Kip1}$. Both endogenous MIF and exogenously added MIF following endocytosis bind to intracellular Jab1. MIF colocalises with Jab1 in the cytosol. MIF inhibits Jab1-, TNF-, and UV-mediated enhancement of AP-1 transcriptional activity as well as Jab1-mediated enhancement of c-Jun DNA binding, but does not interfere with induction of NFkB activity. The enzymatic mutant C60SMIF binds to Jab1 but does not influence AP-1 transcriptional activity. Jab1 activates JNK activity and enhances endogenous phospho-c-Jun levels and MIF markedly inhibits these effects. MIF also counterregulates Jab1-dependent cell cycle processes. MIF potently increases $p27^{Kip1}$ expression in a Jab1-dependent manner. This effect is not due to induction of $p27^{Kip1}$ synthesis but to interference of MIF with Jab1-mediated degradation of $p27^{Kip1}$. Consequently, Jab1-mediated rescue of fibroblasts from growth arrest is blocked by MIF. Our data indicate that Jab1 is the/one of the long sought receptor(s)/binding protein(s) of MIF and that MIF broadly acts to negatively regulate Jab1-controlled pathways and suggest that MIF/Jab1 interaction could provide an important molecular basis for key activities of the unusual cytokine MIF.

13014

Identification and characterisation of CKLiK a novel granulocyte Ca^{2+}/calmodulin-dependent kinase

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Abstract

Human granulocytes are characterised by a variety of specific effector functions involved in host defense. Several widely expressed protein kinases have been implicated in the regulation of these effector functions. We employed a PCR-based strategy to identify novel granulocyte specific kinases. A novel protein kinase cDNA with an open reading frame of 357 amino acids was identified with homology to calcium-calmodulin-dependent kinase I (CaMKI). We have termed it CaMKI-like kinase (CKLiK). Analysis of *CKLiK* mRNA expression in hematopoietic cells demonstrated an almost exclusive expression in human polymorphonuclear leukocytes (PMN). Upregulation of CKLiK mRNA occurs during neutrophilic differentiation of CD34+ stem cells. CKLiK kinase activity was dependent on Ca^{2+} and calmodulin as analysed by *in vitro* phosphorylation of cAMP responsive element modulator (CREM). Furthermore, CKLiK transfected cells treated with ionomycin demonstrated an induction of CRE binding protein (CREB) transcriptional activity compared to control cells. Additionally, CaMK-kinaseα enhanced CKLiK activity. *In vivo* activation of CKLiK was shown by addition of IL-8 to a myeloid cell line stably expressing CKLiK. Furthermore inducible activation of CKLiK was sufficient to induce ERK MAP kinase activity. These data identify a novel Ca^{2+}/calmodulin-dependent PMN specific kinase that may play a role in Ca^{2+}-mediated regulation of human granulocyte functions.

Oral/nasal interferons and cytokines

24006

OROMUCOSAL INTERFERON THERAPY : MECHANISM(S) OF ACTION

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Although oromucosal administration of interferons results in marked antiviral and antitumor activity neither native IFN nor IFN induced proteins are detected in serum or peripheral blood mononuclear cells respectively. The use of transgenic mice expressing a green fluorescent protein reporter gene regulated by an IFN responsive element has shown, however, that IFN activated cells are present in the peripheral circulation 4 hours after the initiation of oromucosal IFN therapy but not at 2 or 8 hours. Differential display analysis has also shown that numerous IFN responsive genes were induced in the lymphoid tissue of the oral cavity at 4 hours together with a number of genes not previously shown to be induced by IFN α including chemokines, proteases associated with antigen processing, and genes involved in lymphocyte activation. In addition numerous novel IFN induced genes were identified which are absent from the data banks (GenBank/EBI). The proteins encoded by 5 of these novel IFN responsive genes have been expressed in an inducible system (Tet-On) and shown to be involved in important cellular processes including apoptosis, and protein degradation. Together these results may explain in part the biological activity of oromucosal IFN therapy.

24001

Low Dose Oral Interferon Therapy: Towards a Mechanism of Action

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Low dose oral interferon (LDO IFN) therapy has been extensively investigated as an alternative administration route for type I interferons. It has been shown to be effective in animal models and in human clinical trials to the phase III level. Despite these demonstrations of efficacy, the mechanism of action of LDO IFN therapy remains largely unknown. Recent studies within our laboratory have shown that LDO IFN therapy reduces white blood cell counts in the spleen and that this reduction is largely restricted to B cells, with minor changes in other immune cell subsets. We propose a model by which the small oral IFN signal is detected and amplified via the dendritic cell network to regional lymph nodes and subsequently to the spleen, causing a mobilisation of B cells to peripheral sites. Type I IFNs have been shown to be a very important part of the innate immune system, both as a first line of defence and in governing the nature and co-ordination of the subsequent acquired immune response. Our new data is integrated with these recent observations and the proposed model supports the notion that LDO IFN mimics a natural defence process based on IFN containing nasal secretions.

24002

INGESTED IFN-α DECREASES NEW MRI BRAIN LESIONS IN RELAPSING-REMITTING MULTIPLE SCLEROSIS (RRMS).

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We asked if 10,000 (low dose-LD) or 30,000 (high dose-HD) IU ingested human IFN-a2a q.o.d. would reduce the number of gadolinium enhancing (Gd) lesions on serial MR brain scans in patients with early RRMS. Entry criteria included early clinically definite RRMS and a baseline Gd T1 lesion on MRI while not on immunosuppressive therapy. Eligible patients were randomized to treatment with placebo, LD or HD IFN-a2a for 9 months with monthly brain MRI scans. Using GEE models, the data suggest a important reduction in new (Gd) lesions, particularly in the LD group. Least squares means analysis (LSM) shows an important treatment effect at month 4 and month 5 in the LD group with a clear decrease in new (Gd) lesions from about 5.6 to 1.0. Cumulative lesion load analysis showed a 30% decrease in the LD group compared to placebo starting at 3 months and continuing until 7 months. Analysis of recall antigen PMNC tetanus toxoid-stimulated TNF-a cytokine secretion showed a significant decrease in the LD group compared to placebo. The reduction of TNF-a correlated with the optimal effect of LD ingested IFN-a by the LSM analysis. The concurrence of maximal decrease of new (Gd) lesions and TNF-a secretion demonstrates that ingested IFN-a, known to decrease TNF-a in EAE, is responsible for the effect on MRI. In addition, we performed an exploratory analysis with the other pivotal MS clinical trials {AVONEX IFN-b-1a trial, IFN-b-1b MRI trial, hrIFN-a2a trial}, comparing our new (Gd) lesion data with their new (Gd) lesion data in a descriptive way. Exploratory analyses suggested that the LD group performed better than the HD group compared to the placebo group in reducing new MRI gadolinium enhancing lesions. Ingested hrIFN-α2a appeared not to be toxic at any dose. These data suggest a potential therapeutic impact of ingested hrIFN-a2a in RRMS.

24007

CYTOKINE REGULATION IN MULTIPLE SCLEROSIS PATIENTS AND SJL MICE AFTER ORAL TREATMENT WITH INTERFERON TAU

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SJL mice and human patients with relapsing-remitting multiple sclerosis were given interferon-tau daily at varying doses. Interferon-tau was diluted in saline solution and administered by oral ingestion. Serum samples were collected at various time points from each subject and stored frozen until day of assay. Each serum sample was assayed for IL-10, interferon gamma, TNF alpha and neopterin levels. Our results will be discussed.

24003

GASTRIC ADMINISTRATION OF ovIFNτ CAN INDUCE BLOOD 2',5'-OLIGOADENYLATE SYNTHETASE IN MOUSE

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IFNτ is a member of type I IFN family. It was reported that oral administration of ovine IFNτ at a high dose (10^5 U/head) prevents the acute and relapsing forms of experimental autoimmune encephalomyelitis (EAE) in mice (Soos J. M. *et al.*, *J. Neuroimmunol.*, **75**, 43-50, 1997). Here, we examined whether ovIFNτ can induce 2',5'-oligoadenylate synthetase (OAS) in mouse and human cells other than ruminal cells in culture. Then, we studied the effect of gastric administration of ovIFNτ into mouse on induction of OAS activity in blood. **[Methods]** ovIFNτ was obtained from Pepgen Co.. OAS induced in cells was detected by Western blot analysis using α-MuOAS or α-HuOAS mAbs. The samples of ovIFNτ was dissolved in 200 μl of 10 % maltose solution were directly administered to the upper part of stomachs of ICR mice (6-week old female) using 20 G oral feeding needles. OAS activities in whole blood were assayed with 2-5A RIA kit (Eiken Chemical Co., Ltd.). **[Results and Discussion]** (1) When L929, HeLa S3 and MDBK cells were treated with ovIFNτ (10^0-10^5 U/ml) for 20 hr, the induction of OAS was observed in each cell line in a dose-dependent manner. (2) ovIFNτ (10^5 U) was given to mice by either gastric administration or i.p. injection. After 24 hr, whole blood was obtained from heart, and blood OAS activities were assayed. The levels of blood OAS induced by gastric administration were almost the same as those induced by i.p. injection. (3) After administration of ovIFNτ (10^3-10^5 U) to the stomach of mice, whole blood was obtained at 12 hr and OAS activities were examined. The results showed that the blood OAS activities increased dose-dependently. This is the first evidence that the gastric administration of IFN can induce blood OAS.

24004

Immunomodulation and tumour cytotoxicity in mice presented orally with plant lectins.

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It is well known that plant lectins such as phytohaemagglutinin (PHA) and misteltoe (ML-1, ML-2, ML-3) are profoundly immunostimulatory. Modulation of the immune system affects the utilisation of nutrients by tissues through a cytokine response, and in addition causes indirectly a modification of endocrine secretion and effects of hormones on tissues. It is possible that these lectins presented orally, in addition to exerting a direct effect on the gut (hyperplasia), also produce a complex series of changes in cytokine response through stimulation of immunocompetent cells found in close proximity to the gut in Peyers patches. These changes, especially evident for IL-2, INF-γ and TNF-α may also influence angiogenesis of the growing tumour, apoptosis and NK-cell activity. In current experiments, mice are presented with lectins orally to reduce the development and growth of a murine non-Hodgkin lymphoma (NHL) tumour. Recent experiments have shown that a daily intake of approximately 10 mg of ML is extremely effective in the treatment of a NHL tumour. Results indicate that ML-1 directly or indirectly causes a reduction in the number of vessels with ensuing impairment of proliferation within the tumour, as well an an augmented T-lymphocyte infiltration into the tumours resulting in increased necrosis. As a result 60 % of the mice showed complete histological ablation of the tumour. Current experiments aim to clarify further the role of cytokines and lymphokines produced from peripheral mononuclear cells as well as the role of NK-cell activity in tumour cytotoxicity in animals presented orally with plant lectins.

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24008

Oral Interferon: Effects on Spleen Cells

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The efficacy of low dose orally administered Type I IFN (LDOA IFN) therapy has been well established in various animal models of disease and in human clinical trials. What remains to be elucidated is the mechanism(s) by which LDOA IFN therapy has its effects. In order to study systemic LDOA IFN effects, our studies have involved only the oral dosing of mice with MuIFNα/β. These studies have revealed dramatic changes in spleen cell populations following LDOA IFN treatment. Specifically, spleen white blood cell (WBC) populations were examined in mice following 7 days of oral treatment with 10 IU MuIFNα/β per day. Spleen weights, total WBC numbers and immune subset analyses were performed in comparison to control mice. No significant difference was found between the spleen weights of treated mice compared to controls. However, reductions in total WBC numbers ranging from 17-30% were consistently observed. The dose response curve for this splenic cell number reduction was U-shaped and optimal between 1 and 10 IU. Furthermore, immune subset analysis of these reductions showed splenic B cell numbers to have been reduced by up to 50%. These effects have been demonstrated in both BALB/c and C57BL/6J mice. A mechanism based on dendritic cells is proposed.

24009

EFFECTS OF ORAL INTERFERON α_{2b} IN LIPOSOMES ON INTERFERON AND IMMUNE SYSTEMS IN ACUTE HEPATITIS B.

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In the past we have reported that liposomes of appropriate composition could protect enclosed substances from the action of intestinal enzymes and detergents and become carriers of biologically active preparations after oral administration. 85 - 90% liposomes with ^{125}I labeled substance given orally is accumulated in liver of experimental animals after 5 min and during 3 hours.

We use liposomes as carriers of oral interferon α_{2b} in acute hepatitis B. In this patients has been detected as remarkably deficiency in IFN - α and IFN - γ producing capacity of leukocytes as various disturbances in humoral and cell-mediated immunity: increase CD19 and decrease CD16 and CD4, CD8 T-lymphocyte phenotypes.

Supposently, in accute hepatitis B Th1 cells response given way to Th2 cells response. Course of oral interferon α_{2b} in liposomes stimulated syntesis of endogenous serum IFN and production firstly of IFN - α and then IFN - γ. Accordingly has been normalized amount of CD4, CD8, CD16, CD19 and ratio CD4/CD8. In other words, oral interferon α_{2b} enclosed in liposomes in acute hepatitis B is able to switch the Th2 cells response over to the Th1 cells.

24010

DESIGNING OF THE DRUGS BASED ON CYTOKINES WITH DETERMINED PHARMACOKINETICAL PROPERTIES

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Using of enteral forms of cytokines is one of the lines of good prospects in correction of immunodeficient states. Some years ago, a tablet enteral drug form for genetically engineered interferon-α2 had been developed. The tablet was protected from gastric enzymes action and disintegrated only in small intestinal. Getting into the small intestinal, the tablets were quickly disintegrated already in the gastric upper sections, and cytokine did not reach the needed quantity of receptors to interferon-α. Using technology of colloid microcapsulation, a prolonged form of genetically engineered interferon-α2 was obtained. Microcapsules of the size of 900 till 20 μm (optimal of 300-400 μm) could be used both as enteral and oral preparations, and for the local application. Controlling of the size and number of grains enabled the transition from pharmokinetics to its formation for each specific drug and its usage in individualized patient treatment. Besides, an opportunity appeared for the use of the drug with addressed properties for target organs and determined pharmacotherapeutical properties.

24011

INGESTED IFN-α MAINTAINS RESIDUAL β CELL FUNCTION IN NEWLY DIAGNOSED IDDM.

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Ingested IFN-α2a prevents IDDM and prevents islet allograft rejection in mice, and can decrease gadolinium enhancements on MR brain scans, a surrogate marker of disease activity, in MS. We examined the efficacy of ingested hrIFN-α2a in maintaining residual β cell function in IDDM in a phase I open label trial. Nine newly diagnosed IDDM caucasian patients < 25 y/o ingested 30,000 IU hrIFN-α2a for one year. The primary outcome measure was a 30% increase of stimulated C-peptide levels after Sustacal® administration at 0-12 months. 7/9 patients demonstrated residual β cell function at 12 months after initiation of ingested IFN-α2a. In four out of the seven type 1 diabetes patients with residual β cell function, anti-GAD antibody titers decreased comparing entry to end of study levels. Examination of serum cytokine data comparing entry to end of study levels show increased IL-4 or IL-10 levels in six of seven patients with residual β cell function. In addition, 4/7 patients were maintained on ingested IFN-α2a for an additional six months and maintained residual β cell function at 18 months after onset. We are currently conducting a phase II randomized, placebo-controlled double-blind clinical trial to establish efficacy of ingested IFN-α2a in newly diagnosed IDDM.

24012

INTESTINAL EPITHELIAL CELL (IEC)-DERIVED CYTOKINE EXPRESSION IN AN EXPERIMENTAL MODEL OF CROHN'S DISEASE (CD)

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The intestinal epithelium represents the primary barrier between gut luminal antigens and the mucosal immune system. CD, a Th1-mediated disorder in which TNF plays a central role, is initiated by a dysregulated immune response to normal gut flora in a genetically susceptible host. SAMP1/Yit mice spontaneously develop chronic ileitis closely resembling human CD with virtually 100% penetrance by 40 wk, and display altered epithelial architecture early in the disease process. The aim of this study was to characterize IEC-derived cytokine profiles in early vs. late phases of disease in SAMP1/Yit mice. IEC isolated from ileal tissues of 10 wk and 50 wk mice were cultured in the presence or absence of TNF (10 ng/ml). IEC lysates and supes were collected for mRNA (after 6 hr) and protein (after 18 hr) levels of the IEC-derived cytokines, IL-1Ra, IL-18, and chemokines, KC and JE/MCP-1. Full-thickness ileal tissues were processed for immunohistochemical staining of IL-1Ra and IL-18. Our results show KC, but not JE/MCP-1 (undetectable), was increased in TNF-induced IEC from 10 wk (stim-6.7±2.8 vs. unstim-2.1±0.3 ng/ml) compared to 50 wk (stim-3.2±1.4 vs. unstim-2.6±1.1 ng/ml) SAMP1/Yit mice. In addition, IEC-derived IL-18 was elevated in 10 wk (stim-1209±104; unstim-1243±72 pg/ml) compared to 50 wk (stim-566±53 vs. 933±140 pg/ml) SAMP1/Yit mice. Western blot analysis confirmed these results and immunohistochemical staining localized both IL-18 and IL-1Ra to the epithelium of the SAMP1/Yit; intense IL-1Ra staining was specifically observed in IEC (M cells) overlying Peyer's patches, but no differences were observed in 50 vs. 10 wk mice. These results support the role that IEC are active participants in mucosal immune responses and IEC-derived cytokines may play an important role in mediating chronic gut inflammation.

Mode of action of interferons

25006

Mode of RNase L Activation: An IFN Regulated Antiviral Enzyme Related to the Unfolded Protein Response Protein, IRE1

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IFNs provide innate immunity to viral infections partly through the 2-5A [2',5'-oligo(rA)] system. IFNs induce a family of 2-5A synthetases that produce a series of 2-5A molecules in response to viral dsRNA. 2-5A activates RNase L producing cleavage of cellular and viral RNA resulting in a potent anti-viral and pro-apoptotic response. An intriguing feature of human RNase L is its homology to the IRE1 proteins that mediate the unfolded protein response in organisms as diverse as yeast and humans. IRE1 is a trans-membrane, kinase/endoribonuclease that is activated by the presence of unfolded proteins in the endoplasmic reticulum. A cross-species RNase L/IRE1 sequence alignment and mutagenesis study of RNase L and IRE1 revealed a related ribonuclease signature sequence. Furthermore, we derived a functional model of activation for RNase L from the comparative study. In the absence of 2-5A, RNase L is believed to exist as a closed, inactive monomer. Binding of 2-5A within a series of ankyrin repeats unmasks the protein/protein interaction sites, leading to dimerization allowing the nuclease domain to stabilize in its active conformation. Removal of the ankyrin repeats from RNase L produced an unregulated enzyme that cleaves RNA in the absence of 2-5A. A lysine to arginine mutation in protein kinase-like domain II of RNase L prevented dimer formation and ribonuclease activity in the complete enzyme. However, the lysine to arginine form of the ankyrin-deleted RNase L remained active, suggesting that the kinase domain facilitates dimerization thus preventing the ankyrin clamp from reforming. These results provide insight into the evolution and function of a molecular pathway of IFN action.

25005

STUDY OF TYPE I INTERFERON SIGNALLING USING CHIMERIC RECEPTORS

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In order to study the impact of the IFNaR1 and IFNaR2-2 receptors of the type I interferon receptor complex, the intracellular and transmembrane domains of these subunits were fused to the extracellular part of the homodimeric EpoR. Although it was noticed in 2fTGH cell lines stably expressing EpoR/IFNaR2-2, that a functional redundancy exists for IFNaR1 subunit in the induced transcription of the *6-16, pkr, 2'5' oas,* and the *mx* genes, IFNaR1 proved to have an essential role in the antiviral cell protection, especially against the VSV virus. In this cell line the ratio of virus production in cells treated by Epo versus IFNα (IFNβ) was 50 (90) for EMCV and 1200 (20000) for VSV.

To trace IFNaR1 domains important in exerting antiviral activity, intracellular parts of the IFNaR1 were C-terminal fused to the EpoR/IFNaR2-2 construct. These studies reveal a functional role of the IRTAM motif in antiviral protection.

Phospho immunoblots of TYK2, JAK1, STAT1, 2 and 3 were performed on 2fTGH cell lines stably transfected with EpoR/IFNaR1 to elucidate the activation status of these chimeric receptors on Epo treatment. Interestingly, IFN-induced tyrosine phosphorylation of TYK2, JAK1 and STAT3 was drastically reduced in cell lines expressing high levels of EpoR/IFNaR1, presumably as a result of the sequestering of an essential cytosolic component. Consistently, also the IFN-induced antiviral protection was impaired in cells expressing high levels of EpoR/IFNaR1.

25002

Differential activation of the common IFNAR1/IFNAR2 receptor complex by interferon subtypes: A comprehensive analysis of gene expression.

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Gene expression profiling analysis was performed to study whether Type I IFNs generate IFNAR-1/ IFNAR-2 receptor complexes with distinguishable signaling properties. IFN-β-1a and IFN-α2b were shown in functional assays to be equally potent on A549 cells but not on human umbilical vein endothelial cells (HUVEC). This finding suggests that differences in affinity of the IFN subtypes for IFNAR-2 cannot explain their potency difference on HUVEC. Dose-dependent gene expression profiles induced in HUVEC by IFN-β-1a and IFN-α2b were determined to address whether the potency difference results from quantitative effects (i.e. how effectively these subtypes induce formation of identical activated receptor complexes in HUVEC), or alternatively from qualitative effects (i.e. that the subtypes form activated receptor complexes which have different signaling properties). GeneCalling analysis, a differential display expression profiling method, provided a comprehensive evaluation of the patterns of gene expression generated by the two IFN subtypes. Approximately 35,000 cDNA restriction fragments were analyzed per experimental condition, and a total of 1,317 differentially displayed bands (exhibiting a ≥ +/- 2-fold change in detection levels) were observed to respond to IFN stimulation. The data show that at 5 ng/ml of these IFNs, the levels of induction and the patterns of gene expression were essentially identical, with < 4% of differentially displayed bands being discretely induced by IFN-β. In contrast, marked differences in gene expression were observed at sub-maximal doses of 50 and 200 pg/ml, with ~30-40% of differentially displayed bands being preferentially or uniquely induced by IFN-β. Thus the results, albeit not ruling out a qualitative difference in receptor activation, are more supportive of a quantitative model in which the binding of these two IFN subtypes generate identical activated receptor complexes in HUVEC, with IFN-β-1a doing so more effectively than IFN-α-2b.

25003

Interferon-α downregulates telomerase reverse transcriptase and telomerase activity in human malignant and non-malignant hematopoietic cells

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Recently, the derepressed expression of the catalytic subunit of telomerase, hTERT, the enzyme that elongates telomeres, has been implicated as an important step in the immortalization process. The exact regulation of hTERT expression, which is the rate-limiting factor for telomerase activity, is at present unclear. As transformed cells seem to be dependent on a constitutive telomerase activity, the availability of inhibitors would potentially be of great value in antineoplastic therapy. Interferons (IFNs) have been successfully used in the treatment of several forms of malignancies, but the underlying molecular mechanisms responsible for the antitumor activity are poorly defined. In this study we have investigated the effects of IFNs on hTERT expression and telomerase activity. We found that IFN-α rapidly (commonly within 4 hours) and significantly downregulates the expression of hTERT and telomerase activity in a number of human malignant hematopoietic cell lines, primary leukemic cells from patients with acute leukemia as well as T-lymphocytes from healthy donors. This effect of IFN-α did not seem to depend on IFN-α-mediated cell growth arrest or alterations in c-myc expression. The finding that IFN induces a repression of hTERT and a decrease in telomerase activity suggests a novel mechanism that may play a significant role in IFN's antitumor action.

25007

Ligand-mediated nuclear chaperoning of STAT1α: The IFNγ paradigm.

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IFNγ is among many extracellular cytokines and growth factors that are known to be translocated to the nucleus in target cells. But little is known about the role of such nuclear translocation of ligands in signal transduction. We have been studying this role using the cytokine interferon-gamma (IFNγ), and provide evidence to support a role for ligand nuclear translocation as a chaperone system for nuclear delivery of STAT1α. IFNγ was found to contain a nuclear localization sequence (NLS), which functioned analogous to the prototypical NLS of the SV40 large T-antigen. A biological and signal transduction defective mutant of IFNγ resulting from deletion of the NLS was restored to full activity and signaling capacity upon reconstitution with the heterologous NLS of the SV40 T-antigen. Thus, nuclear translocation of IFNγ is an intrinsic requirement for IFNγ signal transduction and activity. Intracellular (cytoplasmic) microinjection of antibodies to the NLS of IFNγ inhibited the nuclear translocation of STAT1α induced by extracellular IFNγ. Thus, internalized IFNγ interacts intracellularly to control STAT1α nuclear translocation. Further investigation showed intracellular IFNγ to be present as a complex of STAT1α and Npi-1, which is the nuclear importer of STAT1α. The formation of the STAT1α/Npi-1/IFNγ complex was found to be dependent on the integrity of the IFNγ NLS. No NLS has been identified on STAT1α. Our data suggest that the NLS for STAT1α nuclear import utilizing Npi-1 is provided by IFNγ. IFNγ also induced the nuclear translocation of the α–chain (IFNGR-1) of the IFNγ receptor. The translocation of IFNGR-1 tightly paralleled the nuclear translocation of STAT1α in a dose- and time-dependent fashion. We have previously identified a high-affinity binding site on the cytoplasmic domain of IFNGR-1 for a C-terminal NLS-containing region of IFNγ, which enhances the affinity of JAK2 immediately downstream on the cytoplasmic domain of IFNGR-1. This interaction provides for a framework for activating and recruiting STAT1α into a complex with IFNγ that ultimately interacts with Npi-1 to drive uptake of STAT1α. Other STAT-utilizing cytokines like IL-1, IL-5 have also been shown to contain functional NLSs. It is possible that ligand-assisted nuclear delivery of STATs may also contribute to the signal transduction mechanisms of these cytokines.

25001

AN OBLIGATORY AND ISGF3-INDEPENDENT ROLE FOR STAT2 IN INTERFERON-INDUCED ANTIPROLIFERATIVE RESPONSES

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STAT2 is a critical component of the type I IFN signaling cascade. In response to IFN-α, the heterotrimeric transcription factor ISGF3, comprised of STAT2, STAT1 and the DNA-binding adapter protein, p48/ISGF3γ/IRF-9, is assembled. Within this complex, STAT1 and IRF-9 directly mediate ISRE binding, while STAT2 provides a potent transactivational domain. IFN-α also induces the formation of ISGF3-independent STAT2-containing complexes, namely STAT2:STAT1 and STAT2:STAT3, that can bind to a pIRE. However, the contribution of each STAT protein to DNA binding is unknown. Crystal structures of STAT1:STAT1 and STAT3:STAT3 complexes bound to DNA have shown that, in the context of a STAT dimer, each monomer contacts only half of the palindromic DNA binding site. Moreover, comparison of the primary amino acid sequence of the DNA-binding domain of several STATs with STAT2, reveals that key residues considered to be critical in DNA-binding are conserved in STAT2. This suggests that, in the context of ISGF3-independent STAT2-containing complexes, STAT2 may exhibit specific DNA-binding activity.
To address the role of STAT2-DNA binding in mediating IFN-inducible responses in target cells, we generated a putative STAT2-DNA binding mutant cDNA, in which only the bases encoding the two glutamate (E) residues within the VTEEL sequence of the putative STAT DNA-binding domain of STAT2 were mutated to bases encoding alanines. Mutant cDNA was introduced by transfection into U6A cells lacking STAT2, resulting in U6A-2M cells that expressed the mutant STAT2 protein. Comparative studies were undertaken to assess the sensitivity of U6A, U6A-2M and cells reconstituted with an intact STAT2 (U6A-2), to IFN-inducible responses. Whereas the U6A-2 cells were fully responsive to IFN-inducible antiviral and growth inhibitory activities, both the U6A and U6A-2M cells failed to exhibit IFN-inducible growth inhibition. U6A, U6A-2 and U6A-2M cells exhibited IFN-inducible STAT1 and STAT3 activation, as well as homo- and hetero-dimer complex formation and pIRE binding. In contrast to the U6A cells, which, as expected, did not exhibit IFN-inducible ISGF3 activation, comparable levels of IFN-inducible ISGF3 activation and ISRE-DNA binding activity were observed in the U6A-2 and U6A-2M cells.
Viewed altogether, these data identify an obligatory and ISGF3-independent role for STAT2 in mediating IFN-inducible antiproliferative activity.

25004

B CELL ACTIVATION IN THE PRESENCE AND ABSENCE OF TYPE I IFN

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B cell activation via B cell receptor (BCR) triggering comprises induction of B cell proliferation, upregulation of various cell surface molecules including B7-2 and CD69, and immunoglobulin secretion. In absence of BCR triggering type I interferons (IFN) binding to the IFN receptor of naïve B cells can upregulate the expression of B7-2, CD69, and other cell surface molecules, however, immunoglobulin secretion is not induced. After 10 h incubation of lymphocytes with 2500 iu/ml IFN-β we observed a >10-fold increased CD69 expression and a 3-fold increased B7-2 expression on IFN receptor competent B cells, whereas IFN receptor deficient B cells did not show such effects. Currently we are studying BCR mediated activation of IFN treated and untreated B cells *in vitro*. To study the influence of IFNs on B cell activation *in vivo* we generated conditional gene targeted mice devoid of IFN receptor expression on B cells. Such mice showed the desired phenotype of IFN unresponsiveness on B cell, but not on other tissues. Currently the conditional IFN receptor deficient mice are analyzed under conditions of infection with vesicular stomatitis virus (VSV), a virus inducing an IFN response and a neutralizing antibody response which are both required to protect mice against lethal paralytic disease.

25008

PIG TROPHOBLAST INTERFERONS : IFN-DELTA IS PARTICULARLY EFFICIENT TO DEPOLARIZE ENDOMETRIAL CELLS

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The pig conceptus (embryo plus trophoblast) is the source of quite unusual interferon productions : at the time of implantation, the embryonic trophectoderm secretes large amounts of IFN-γ, and in lesser amounts IFN-delta, a recently described specie of type I IFN.
In view of possible effects of one or both of these IFNs on the endometrial epithelium, the effects of IFN-γ and IFN-δ on endometrial cells was studied, under two aspects : apico-basal polarity, and susceptibility to antiviral effect. Two spontaneous endometrial epithelial cell lines were isolated, one (EL) with more glandular phenotype, the other (ELE) probably luminal by origin. Both cell lines were cultured on microporous inserts, on which both cell lines developped after some days an apico-basal polarisation, measured by the appearance of transepithelial electrical resistance and potential. Recombinant IFN-γ and IFN-δ were added alone or in combination for 24 hrs, from either the apical or basal side of inserts. Type I IFN-delta was the only one that had significant depolarizing effet on luminal ELE cells, being more efficient from the apical side. On the glandular EL cell line, 24-h treatment by either IFN-δ or -γ led to depolarization, provided IFNs were applied from the basal side, which suggests the absence of IFN receptors on the EL apical membrane. In this effect, IFN-δ was much more efficient than IFN-γ at an equal antiviral dose. Assay of antiviral activity in the same experimental model gave concordant results. These results not only show that the IFN-δ is particularly active to disrupt/depolarize epithelial barriers, but give some clues to the respective roles of embryonic type I and II IFNs in pig early pregnancy.

Cytokines and interferons in cancer

26006

RECONSTITUTION OF ENDOGENOUS IFN-α THROUGH DOWNREGULATION OF TGF-ß EXPRESSION BY rh-IFN-α IN HAIRY CELL LEUKEMIA

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As previously shown, a deficiency in the production of haematopoietic growth factors and overexpression of TGF-ß1 are found in hairy cell leukaemia (HCL). In spite of the established therapeutic role of rh-IFN-α in this disease, the mechanisms leading to its beneficial effect are not completely clarified and there is no information on IFN-α gene expression in this disease. Therefore, we investigated the pattern of IFN-α gene expression and protein production in HCL and the possible involvement of TGF-ß in the regulation of this process. Expression of IFN-α mRNA was assessed by RT-PCR analysis in peripheral blood mononuclear cells (PBMC) under basal conditions and upon induction with rhIFN-α and polyionosinic-polycytidylic acid (poly I:C). IFN-α concentrations were measured by immunoassays and intracellular IFN-α was evaluated by FACS analysis. Results showed that, in contrast to HD, freshly isolated PBMC from untreated HCL patients did not express IFN-α mRNA while IFN-α transcripts were found in patients who were under rhIFN-α therapy. Plasma of untreated patients contained non or extremely low levels of IFN-α as compared to treated patients and HD. *Ex vivo* treatment of PBMC with rhIFN-α or poly I:C resulted in a remarkable upregulation of IFN-α at the mRNA and protein level. While inhibition of IFN-α transcription was found after exposure of PBMC to serum from untreated patients. Also exposure of the cells to TGF-ß led to inhibition of IFN-α mRNA expression and protein production. Neutralising anti-TGF-ß antibodies enhanced the expression of IFN-α at mRNA protein level. These results demonstrate a deficient production of IFN-α in HCL and that the therapeutic effect of rh-IFN-α might be through the induction of endogenous IFN-α production and down regulation of TGF-ß expression.

26004

LONG TERM EVALUATION OF THE USE OF NATURAL LEUKOCYTE OR RECOMBINANT INTERFERON ALPHA-2B IN THE TREATMENT OF MYCOSIS FUNGOIDE. COMPARATIVE, RANDOMIZED, DOUBLE BLIND STUDY.

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A randomized, double blind study comparing treatment with natural leukocyte and recombinant interferon alpha-2b (HEBERON ALFA N and HEBERON ALFA R, respectively, Heber Biotec, Havana) was performed. Patients were included if they had clinical, histologic and immunohistochemical diagnosis of mycosis fungoide (MF), gave their written, informed consent to participate and had not received specific treatment for 3 months. The dose was 9 x 10^6 IU 3 times per week, intramuscularly, during one year and then 6 x 10^6 IU twice per week until progression. 37 patients have completed the first 6 months treatment period. The table below shows the main results. A high response rate was obtained in both groups, confirming that IFN alpha treatment is beneficial in MF. Moreover, most non-responders had stable disease. Overall progression-free rate was more than 75 % at 36 months of follow-up. Response rate to natural leukocyte IFN was always higher than to recombinant IFN, statistically significant at 12 and 18 months. Two of the non-responders from the recombinant IFN alpha-2b group, developed neutralizing-antibodies. The rest of the adverse reactions profile was similar for both IFN preparations but it seemed better for leukocyte IFN (not significant).

Overall response (%) rate at different times

	6 months	12 months	18 months	24 months	36 months
Leu-IFN	11/17 (65)	11/15*(73)	10/13*(77)	8/12 (67)	5/9 (56)
Rec-IFN	11/20 (55)	7/17 (41)	7/16 (44)	8/16 (50)	5/12 (42)

26002

IL-4 INDIRECTLY SUPPRESSES IL-2 PRODUCTION IN THP HUMAN T CELLS BY MACROPHAGE PRODUCED PPARγ LIGANDS

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Interleukin-2 (IL-2), primarily a product of the Thp and Th1 helper cells, controls T cell survival, clonal expansion, and functional differentiation. Previously, we have shown that peroxisome proliferator-activated receptor γ (PPARγ), a unique member of the nuclear receptor family, blocks IL-2 production by inhibiting NFAT-mediated IL-2 gene transcription in human T cells. Activated PPARγ physically associats with NFAT, blocking nuclear translocation and IL2 promoter activity. This represents a novel mechanism and function of PPARγ in T cell biology. The respective development of either Th1 or Th2 cells is mediated by the effects of cytokines directly on Thp cells. Recently, IL-4, principally produced by Th2 cells, has been shown to induce "new" potential PPARγ ligands such as 13-HODE and 15-HETE by the IL-4 dependent induction of 12/15-lipoxygenase in macrophages, suggesting that IL-4 may indirectly affect the production of IL-2 by Thp helper cells and block the subsequent development of Th1 cells by interfering with IL-2 transcription. We have analyzed the effects of several IL-4/macrophage induced 12/15 lipoxygenase products on the production of IL-2 by Thp human T cells. Both 13-HODE and 15-HETE potently blocked IL-2 production/transcription by freshly isolated human T cells. Using a PPARγ transfected Jurkat cell line, we have demonstrated the specificity of these lipoxygenase products on the inhibition of NFAT, the IL-2 promoter reporter, and endogenous expression of IL-2. Our data provide a mechanism whereby the Th2 cytokine IL-4 may indirectly affect Thp function via macrophage products of the 12/15-lipoxygenase pathway. Macrophage produced PPARγ ligands function to modulate immune responsiveness by affecting the PPARγ in both lymphocytes and macrophages by interfering with activated transcription factors such as NFAT, NFκB and AP-1.

26005

DNA IMMUNIZATION INDUCES SPECIFIC PATTERNS OF CYTOKINES AND CHEMOKINES IN THE SKIN: IMPLICATIONS FOR TUMOR IMMUNITY.

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The ability to overcome immunologic tolerance to self-antigens represents a major step in the development of effective vaccines for human cancers. By utilizing xenogeneic DNA coding for melanosomal differentiation antigens, we have shown that tolerance can be broken, resulting in protection from syngeneic tumor challenge in a mouse model system for melanoma. Depending on the antigen used, DNA vaccination induces antibodies or $CD4^+$ and $CD8^+$ T-cell responses against tumors. To further understand the mechanisms of Th1 or Th2-directed responses, we have examined early events following DNA immunization. Measurements of cytokine and chemokine levels in the skin of mice immunized with either mouse or human $gp75^{TRP-1}$ (tyrosinase-related protein-1) or TRP-2 demonstrate a differential expression of the various cytokines over time and with the use of different genes. The early response is characterized by high levels of inflammatory molecules such as IL-1β, IL-6, TNFα, RANTES, MIP-1α and MCP-1. Precursor Th1 cytokines, IL-12 and IL-18, appear within 24-48 hours concomitant with IFNγ production, while the IL-4 response peaks at 48 hours and is maintained beyond 192 hours, when other cytokines have returned to baseline. In addition, use of an immune adjuvant, GM-CSF DNA, induces measurable levels of GM-CSF protein both in the skin and serum. The GM-CSF peak is also associated with high levels of MIP-1α and MCP-1. The pattern of cytokines produced in response to DNA immunization and their expression over time have implications for our understanding of early events in the immune response. They give an indication of the cells that are being recruited from the circulation and provide models to develop new approaches to cancer vaccines, including the use of cytokine genes as adjuvants.

26003

NF-κB REGULATION OF RENAL CARCINOMA BY IFN-ALPHA AND RESPONSE TO CHEMOTHERAPY

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Objectives: To investigate a possible tumor-resuscitating role of NF-κB in the treatment of renal cell carcinoma with immunochemotherapy (IL2, IFNα, cytostatics). **Material:** Primary cultures of renal cell carcinoma (RCC) were derived after surgical removal of tumors or metastases. Baseline and induced NF-κB activity (by TNFα) was determined using a beta-Gal / NF-κB reporter plasmid. RCC were then treated with cytokines (IL2, IFNα), inhibitors of NF-κB signaling (CAPE, BAY, PDTC) or transfected with NF-κB expression plasmids (either activating or blocking NF-κB), and subjected to treatment with cytostatics (doxorubicin, 5-fluorouracil, gemcitabine, caelyx®). Cell killing was determined by video-microscopy or conventional proliferation assays. **Results:** RCC fall into three groups with respect to NF-κB response: **a)** regular (low baseline, inducible by TNFα, response suppressed by IFNα); **b)** stable (no regulation by TNFα and/or IFNα); **c)** non-suppressible (induction by TNFα not reversed by IFNα). Preteatment of RCC with IFNα renders selected RCC more susceptible to TIL or cytostatics attack compared to untreated cells. The same effect could be observed by interfering with NF-κB through either expression plasmids or NF-κB inhibitors. However, only in rare cases did pretreatment induce response in primarily unresponsive cell lines. Effect of IFNα pretreatment and of interference with NF-κB coincided in a number of the responsive tumors. **Conclusions:** IFNα pretreatment and interference with NF-κB signalling share common effects in the modulation of RCC response to cytotoxic effectors. Contrary to our expectations, NF-κB is not a common mediator of tumor unresponsiveness to induction of apoptosis. However, as the respective tests are easily carried out close to the treatment site we consider making an assessment tumor sample response before initiating treatment. Herefore, we are currently correlating our laboratory data with clinical outcome of patients submitted to immunochemotherapy.

26007

MECHANISMS OF THE DIVERSE ANTI-TUMOR EFFECTS OF TUMOR CELL-ASSOCIATED IL-1 ALPHA AND IL-1 BETA

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IL-1 consists of a family of two proteins, namely IL-1α and IL-1β, which overlap in their biological activities, in their recombinant form, and bind to the same receptors. Their differential localization in the producing cell may determine their physiological functions; IL-1α is active as a cytosolic precursor and as a membrane-associated form, whereas IL-1β is active only as a secreted product. Previously, we have shown the anti-tumor and immunotherapeutic potential of fibrosarcomas transfected with the precursor of IL-1α. In this study, the same violent fibrosarcomas were transfected with IL-1β-encoding cDNA constructs; the mature form of IL-1β and the mature form of IL-1β linked to a signal peptide to allow active secretion through the ER-Golgi pathway. To our surprise, IL-1β transfectants did not lose their tumorigenicity and were even more malignant than the violent parental tumor cells. This is in contrast to the IL-1α transfectants, derived from the same parental fibrosarcoma cells, which completely regress. The marked differences in the tumorigenicity of IL-1α versus IL-1β transfectants stem form differences in the immunogenicity of the IL-1 expressing cells; tumor cell-associated IL-1α is a strong adjuvant, whereas IL-1β expression by the malignant cells does not affect their low immunogenicity. In addition, tumor cell-associated IL-1α and IL-1β differentially affected molecules that are involved in tumor progression. Thus, IL-1α expression in the fibrosarcoma cells down-regulated metalloproteinase (MMP)-9 and MMP-2 expression, whereas in IL-1β transfectants these genes were unregulated, as compared to the violent cells. The results substantiate the hypothesis the diversity in the biological functions of the IL-1 molecules can be envisioned only in the context of the producing cells or its direct microenvironment and not when the recombinant cytokines are exogenously added to in vitro or in vivo experimental systems. The complex effects of IL-1α and IL-1β on the tumor cells and the tumor microenvironment are being elucidated in our lab.

26001

LEC-EXPRESSING TSA TUMOR CELLS ARE THE MOST IMMUNOGENIC AMONG THOSE ENGINEERED TO RELEASE CYTOKINES AND CHEMOKINES.

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Because of the release of chemokines many solid tumors of epithelial origin are infiltrated by a significant number of host immune cells, mainly of mononuclear lineage. The aim of our work is to evaluate whether constitutive expression of chemokines influences the tumorigenicity of TSA cells, a poorly immunogenic line established from a spontaneous adenocarcinoma of a BALB/c female mouse. The ability of TSA transfected cells compared with that of parental cells (TSA-pc) to give rise to solid tumors in syngeneic mice was evaluated. The tumor specific immune memory elicited by various TSA transfectants and the reaction mechanisms involved have been studied functionally and through morphological analysis. TSA-pc grow progressively, kill both nu/nu and syngeneic BALB/c mice and give rise to lung metastases. TSA cells engineered to release CXC chemokines are rejected by a minority of mice only. TSA cells engineered to release CC chemokines (MIP-4, MPIF-1, MCP-4, and ELC) are rejected by 12-30% of mice only. By contrast striking results were obtained with TSA cells engineered to release LEC (TSA-LEC cells). They are still able to grow in nu/nu mice, but are promptly rejected and display a marginal metastastic phenotype in BALB/c mice. Rejection **a)** is associated with a marked T-lymphocyte, granulocyte, macrophage and dendritic cells (DC) recruitment; **b)** is impaired by depletion of both $CD8^+$ lymphocytes and polymorphonuclear leukocytes (PMN). Most important, rejection elicits an antitumor immune memory that takes place quickly. Six days after TSA-LEC challenge 75% of BALB/c mice were already resistant to a contralateral TSA-pc challenge. DC at tumor site probably play a crucial role in this fast onset of systemic immunity. The memory elicited by TSA-LEC is associated with anti-TSA antibody titers that are higher than those observed after vaccination with TSA-IL-4, TSA-IL-10 and TSA-IL-12. Moreover, in vitro experiments disclosed that the triggering activity of LEC is even more divided, since it activated monocytes to kill TSA-pc cells and to release RANTES and MCP-1. The ability of LEC to markedly improve recognition of poorly immunogenic cells by promoting antigen presenting cell-T cell cross-talk suggests that it could be a useful component of antitumor vaccines.

26023

Immunomodulation based cancer therapies with Adriamycin plus cytokines

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Previous reports from this laboratory have shown that Adriamycin (DOX) has multiple immunomodulating effects in C57BL/6 mice (Ehrke and Mihich, Immunopharmacology of anticancer agents, In: *Immunopharmacology Reviews*, Vol. 2, J.W. Hadden and A. Szentivanyi, eds., Plenum Pub. Corp. NY, 1996, Chapter 4, pgs. 103-127). In these mice, implanted s.c. with 10^5 syngeneic EL4 lymphoma cells, curative effects could be obtained with moderate doses of DOX plus prolonged treatments with low doses of IL2 or TNF. It should be noted that 10 EL4 cells implanted s.c. into untreated C57BL/6 mice will ultimately kill all the hosts. The curative effects of the combination treatments were dependent on an augmentation of antitumor host responses based on T cells function. The combination treatment induced long-term survivors which exhibit life-long specific T cell dependent immunological memory against EL4. In the same strain of mice, combinations of DOX plus TNF also had curative effects against mammary adenocarcinoma EO771; modulation of both NK and T effector cells correlated with the therapeutic response. The overall results obtained indicated that combined treatments with an anticancer drug and certain cytokines can exert therapeutic effects which are dependent on the immunomodulating effects induced by both combinants.

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26011

INTERFERON GAMMA INDUCED APOPTOSIS IN TRANSITIONAL BLADDER CANCER CELLS: RELEASE OF CYTOCHROME-C AND ACTIVATION OF CASPASE-3, 8, AND 9.

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Intravesical instillation of *Bacillus Calmette Guerin* (BCG) is the most effective treatment for superficial transitional cell carcinoma (TCC) of the urinary bladder. However the mechanisms of antitumor action of BCG are unknown. BCG treatment induces many cytokines and several studies have indicated that IFN-γ is one of the critical cytokines produced in BCG response group. To test whether IFN-γ can induce apoptosis in transitional carcinoma cell lines (TCC), we treated four TCCs with 500 U/ml of IFN-γ and all cell lines showed typical morphological characteristics of apoptosis after 3 days. We used 5637-cells for further experiments. Trypan blue exclusion assay showed approximately 75% cell death after treatment with 500 U/ml of IFN-γ at 96h. DNA fragmentation analyses also confirmed the apoptotic cell death induced by IFN-γ treatment. About 70% inhibition of apoptosis was observed with a general caspase inhibitor (ZVAD). Three to four fold mRNAs induction of Caspase 1, 3, 7, 8, and 9 by RNase protection assay, three fold induction of enzymatic activities of caspase 3, 8, and 9, and release of cytochrome-c in the cytosol were detected in IFN-γ treated cells compared to untreated control cells. Understanding the molecular mechanisms of IFN-γ induced apoptosis in TCCs may help in future to design better therapeutic approaches against bladder cancer.

26012

MALIGNANT PLEURAL EFFUSIONS AND THE THERAPEUTICAL IMPACT OF GM-CSF INFUSION: A CLINICAL AND ULTRASTRUCTURAL ANALYSIS

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Malignant pleural effusion is a complication of cancer. Systemic chemotherapy is effective in small percentage of patients. Attention has been focused on local methods of control. In our study we used GM-CSF to investigate its effect on local immune mechanisms and clinical response.
The first male patient, 60 years old, with lung cancer and malignant pleural effusion entered the study. The production rate of the effusion was 2000 ml in 4 days. After infusion of 300 μg of GM-CSF the 10th day paracentesis yielded 500 ml of effusión. Effusion samples were obtained before and after GM-CSF infusion, centrifuged and processed for ultrastructural analysis.
Before treatment both neutrophils and lymphocytes presented few number of microvilli and increased vacuolization. The malignant cells were poorly differentiated. After treatment a decrease of neutrophils and lymphocytes were observed along with an increase in their microvilli. Apoptosis was a prominent phenomenon. Malignant tumor cells also presented with strong apoptotic tendencies and secondary necrosis.
Our data showed that the local therapy with GM-CSF achieves a decrease in the amount of effusion and an increase of the interval between two consecutive paracenteses. Furthermore, the activation triggered by GM-CSF induces apoptosis. Consequently, the depletion of neutrophils and lymphocytes observed after GM-CSF infusion can be attributed to apoptosis. This was also prominent in tumor cells.

26013

MECHANISMS INVOLVED IN THE ANTI-TUMOUR ACTIVITY OF IFN-γ IN HUMAN OVARIAN CANCER.

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IFN-γ has some anti-tumour activity in human ovarian cancer and inhibited proliferation and induced apoptosis in three of four ovarian cancer cell lines *in vitro* and in 11/14 freshly isolated ascites samples obtained from ovarian cancer patients. We have shown that a continuous interaction with the IFN-γ receptor, together with a sustained induction of p21 and IRF-1, is associated with growth inhibitory and apoptotic effects of IFN-γ in ovarian cancer cell lines. Using a cytokine cDNA array we have shown that as IFN-γ sensitive cells become committed to cell death, members of the TNF superfamily such as TNF inducible protein TSG-6 and CD27BP (Siva) are induced. A further understanding of the mechanisms of action of IFN-γ in ovarian cancer may help in determining which patients might benefit from this therapy.

26014

EFFICACY OF TYPE I INTERFERONS IN J2E CELL PROLIFERATION, DIFFERENTIATION AND DEVELOPMENT OF ERYTHROLEUKEMIA

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The J2E erythroleukemic cell line, immortalized at the proerythroblast/basophilic erythroblast stage of development, was generated by transformation of erythroid precursors with the *raf* and *myc* oncogenes. In culture, J2E cells stimulated with erythropoietin (epo) show increased proliferation, enhanced viability, production of hemaglobin, and a proportion of cells enucleate to form mature reticulocytes. In mice, J2E cells produce a rapid, fatal erythroleukemia characterized by hepatosplenomegaly and severe anaemia. We have investigated the effects of type I interferons (IFNs) alpha 1, 2, 4, 5, 6, 9 and beta on J2E cells *in vitro* and *in vivo*. Type I IFNs differentially suppressed epo-induced J2E cell proliferation. In contrast, a panel of IFN subtypes were able to enhance J2E cell differentiation. *In vivo* we investigated prophylactic immunizaton of nu/nu mice with type I IFN naked DNA and the development of J2E-induced erythroleukemia. Progression of erythroleukemia was monitored by the development of anaemia and splenomegaly for the different IFN subtypes. Type I IFNs differed in their capacity to restrict the development of erythroleukemia in nu/nu mice. This work highlights the need for appropriate use of type IFN subtypes in therapeutic applications and invites the notion of combination IFN subtype treatment.

26025

MODULATION OF INVASION OF PRIMARY RENAL CARCINOMA CULTURES INTO AN ARTIFICIAL STROMA BY IL6 AND TGF-beta1: EXPRESSION OF CATHEPSIN B AND L AND MMP-2 AND MMP-9

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Objectives: To investigate a possible role of the cytokines IL-6 and TGF-beta1 in the process of invasion of renal cell carcinoma (RCC) into an artificial stroma. To identify modulation of the proteases Cathepsin B and L and MMP-2 and MMP-9, by the cytokines. **Material:** Primary cultures of RCC (n=15) and control tissue (n=4) were derived immediately after surgical removal of tumors and / or metastases. After testing for cytokeratin (exclusion of fibroblasts), CellTracker® (Molecular Probes)-labeled RCC were plated onto a cushion of fibroblasts in collagen-I. During 10-14 days of culture, cells were re-fed every second day with medium containing IL-6 (10ng/ml), TGF-beta1 (10ng/ml) or anti-IL6 (2mkg/ml, neutralizes up to 40ng/ml IL6). By serial sectioning and immunostaining, pattern of invasion and expression of proteases were assessed. A parameter derived from the area / circumference ratio (Q=1 for circle, larger for all other forms) of the tumor cell compartment was used to determine invasive growth. **Results:** Control tissue renal epithelium forms a straight layer atop the stroma or forms a lump of cells surrounded by the collagen / fibroblast mix. No invasion is observed and expression of proteases is weak. RCC cells behave variably. Typically, in non-stimulated cultures, a compact block of proliferating tumor- and stroma cells is observed, however, no invasion occurs (Q≈1). However, in certain tumors IL6 leads to a diffuse invasion of single tumor cells (Q≈5.6), while TGFb1 yields both proliferation of tumor and stroma cells and invasion of string-like tumor cell aggregates (Q≈3.3). Expression of Cathepsins B and L was high and localized to the RCC cells, while that of MMP-2 and -9 usually was weaker and diffuse. **Conclusions:** The cytokines, IL6 and TGFb, that can be expressed by RCC, modulate the invasive behaviour of these tumors. Currently, we investigate possibilities to counteract this effect with antibodies and protease inhibitors.

26031

ACTIVATION AND REGULATION OF THE IRF-4 TRANSCRIPTION FACTOR IN HUMAN T-CELL LEUKEMIA VIRUS TYPE I (HTLV-I) INFECTED T-CELLS

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The Human T cell Leukemia virus type I (HTLV-I) is the etiological agent of a number of diseases, including adult T cell leukemia (ATL) and an inflammatory neurological demyelinating syndrome called HTLV-I-associated myelopathy/tropical spastic paraperesis (HAM/TSP). HTLV-I infects and transforms $CD4^+$ T cells both *in vivo* and *in vitro*, a process which requires the dysregulated expression of a number of cellular genes involved in cellular activation and proliferation, cytokine signalling and apoptosis. A novel member of the Interferon Regulatory Factor family of transcription factors, IRF-4, was demonstrated to be constitutively produced in HTLV-I infected cell lines and PBL derived from ATL patients. IRF-4, which is essential for the function and homeostasis of mature B and T lymphocytes, is transiently expressed in anti-CD3 and PMA/ionomycin stimulated T lymphocytes but not in continuous non-HTLV-I infected T-cell lines. It was recently shown that c-Rel, a member of the Rel/NF-κB family of transcription factors, was necessary for IRF-4 gene expression in B and T lymphocytes. In an effort to further characterize the requirements for IRF-4 activation in the context of HTLV-I infection, we have performed immunoblot, DNA binding and transient co-expression assays to identify several DNA binding domains that interact with NF-κB and NF-AT transcription factors to drive IRF-4 gene expression in T cells.

26035

ProFusion™: A POWERFUL TOOL FOR RAPID DISCOVERY OF HIGH AFFINITY BINDERS TO CYTOKINE AND CYTOKINE RECEPTORS

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ProFusion™ is a novel technology that leads to the rapid discovery of interacting peptides and proteins to molecular targets of biological and pharmaceutical interest. Because the coding information (mRNA) is covalently linked to the protein, this technology makes it possible to enrich for binders by multiple rounds of selection from libraries consisting of a large number of diverse molecules (10^{13}-10^{14}). We are applying this technology to identify high affinity binders to dozens of cytokines and cytokine receptors. From a library of antibody mimics, we have isolated low nanomolar binders to a number of cytokines and receptors involved in cancer, inflammation and angiogenesis. These antibody mimics will be leveraged to elucidate the role of such cytokines and receptors in these disease states. The robustness and in vitro nature of ProFusion™ make it a powerful tool for rapid discovery (3-4 weeks) of molecular interactors on a routine basis.

26021

IMPAIRED ANGIOGENIC BALANCE AND SUPPRESSION OF TUMORIGENICITY IN HELA CELLS CHRONICALLY EXPOSED TO INTERFERON-α

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We have previously reported that IFNα-chronic treatment during 41 days induced a partial phenotype reversion on HeLa cells along with a down-regulation of HPV18 mRNA levels. However, tumorigenicity of these cells in nude mice was unchanged. Interestingly, after one-year of IFNα-chronic exposition, HeLa cells failed to induce s.c. tumors when injected into nude mice. In such experimental conditions both HPV18 DNA integration pattern and viral DNA copy number present in HeLa cells remained intact in the nontumorigenic phenotype cells. As result of the treatment with IFNα, HeLa cells rendered more resistant to lysis mediated by activated natural killer cells *in vitro*. Furthermore, IFNα-chronic treatment was able to induce VEGF and decrease bFGF mRNA expression, what suggest a potential effect on the angiogenic behavior of these tumoral cells. Thus, long-term treatment of HeLa cells with IFNα, can accomplish a reversion of the malignant phenotype by a sequential multistep mechanism, in which the antiangiogenic effect of IFNα could be one of the contributing events.

26032

DOWNREGULATION OF TGF-ß1 EXPRESSION AND INDUCTION OF APOPTOSIS IN HUMAN TUMOR CELLS BY BISPHOSPHONATE (ALENDRONATE)

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Transforming growth factor-beta (TGF-ß) is present at high concentrations in bone matrix and plays a regulatory function in tumor growth and metastasis. On the other hand, Bisphosphonates (BP) are potent antiresorptive compounds with a potential beneficial effect in reducing skeletal metastasis in patients with cancer. However, there in no clear information on the mode of action of BP on tumor cells. Therefore, we investigated the effect of BP on human tumor cells and on TGF-ß expression. Human osteosarcoma cells (HOS) were treated with TGF-ß1 or with BP-Alendronate (ALN). Proliferation assays showed that TGF-ß has an enhancing effect on cell proliferation while a potent inhibitory effect was observed upon exposure to ALN. Alendronate-treated cells retracted, became round, lost adhesion to the surface and fragmented. Hoechst, Annexin V and PI staining showed the typical features of apoptotic cell death. DNA extraction and gel electrophoresis revealed DNA fragmentation and laddering confirming cell death by apoptosis. Since interference with cell adhesion may lead to cell death, HOS cells were treated with ALN oncentrations and before or after adhesion to the culture plates. Results confirmed that the effect of ALN was not due to an interference with cell adhesion but rather due to a direct effect on tumor cells. Interestingly, RT-PCR analysis and immunoassays showed that the growth inhibitory effect of ALN on the tumor cells is associated with downregulation of TGF-ß1 expression at mRNA and protein level. TGF-ß could significantly protect the cells from the apoptotic effect of ALN. These results indicate that Bisphosphonate (ALN) inhibits proliferation and induces apoptosis in human osteosarcoma cells through a mechanism which may involves downregulation of TGF-ß1 expression, thereby presenting a novel approach in cancer therapy.

26034

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INTERLEUKIN-6 AND ONCOSTATIN M SENSITIZE FOR TNF-INDUCED CELL DEATH

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Interleukin-6 (IL-6) was described to be a cytokine acting on cervical carcinomas in an autocrine manner and to have anti-apoptotic properties in other cell types. In this study we investigated the mechanisms underlying the response of cervical carcinoma cells to IL-6. We show that IL-6 is in fact highly expressed in cervical carcinoma cells *in vitro* and *in vivo*, while absent in non-malignant transformed keratinocytes. However, in contrast to the non-malignant cells, carcinoma cells did not respond to IL-6 with STAT3 activation, a hallmark of IL-6 signaling, arguing against an autocrine stimulation loop. While all tested cells expressed the signal-transducing ß-chain of the IL-6 receptor (gp130) and also STAT3 at comparable levels, the ligand-binding IL-6Rα-chain (gp80) was hardly or not detectable in the carcinoma cells. Only the addition of soluble gp80 (sgp80) rendered the carcinoma cells responsive to exogenous and also to endogenously produced IL-6 as demonstrated by STAT3 activation. Carcinoma cells having responded to IL-6 showed enhanced susceptibility to TNF-mediated cell death. Sensitization to TNF apoptosis was not only induced after IL-6 stimulation but also after application of the STAT3-activating cytokine oncostatin M. Our data show that STAT3-activating cytokines sensitize HPV-transformed keratinocytes and also other cell types for TNF-mediated cell death. This may help to explain why cervical carcinoma cells are protected from autocrine stimulation via the IL-6 cytokine.

26033

The in vitro anticancer drug cytotoxicity on MDR$^+$ and Bcl-2$^+$ tumor cells: the enhancement by human recombinant α-2- interferon (Roferon A, Rof A).

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Now it is very good known, that some cytokines, especially interferons may enhance the antitumor effects of chemotherapeutic agents. In our recent studies it has been shown the enhancement of the cytotoxic effects of vincristine, doxorubicine, etoposide, mitoxantron and some other drugs on the different cancer cell lines, such as K-562, colon carcinomas HT-29 and SW-480 by 200 - 2000 IU/ml of Rof A. In this study we investigated the dependence of such Rof A action from the expression of two genes, related with cell sensitivity to the drug cytotoxicity - mdr-1 and bcl-2 genes. We did not find the difference between the normal HT-29 cells and their MDR-expressing countreparts in the Rof A-induced enhancement of theit sensitivity to the cytotoxicity of doxorubicin and vincristin. Moreover the cytotoxicity of MDR-independent drug 5-fluorouracil (5-FU) proved to be enhanced by Rof A. We found also the enhancement of cytotoxicity of doxorubicin and 5-FU both in the wild type of HeLa cells and in their bcl-2-transfected couterpart by 500 IU/ml of Rof A, but much more strong in bcl-2-expressing cells. Drug cytotoxicity enhancing activity of Rof A is much more strong on the low drug concentration, inducing of apoptotic cell death, than on necrosis-inducing high doses. Rof A itself are not toxic on target cells. Now we study the influence of Roferon A on the expression of bcl-2 gene in this and some others tumor cells systems and on the apoptosis induction.

26028

INTERFERON-β INDUCES S-PHASE SLOWING VIA UP-REGULATED EXPRESSION OF PML IN SQUAMOUS CARCINOMA CELLS

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Type I Interferon (IFN) and all-trans retinoic acid (RA) inhibit cell proliferation of squamous carcinoma cell lines (SCC) by inducing apoptotic cell death. Examinations of growth-affected cell populations show that both cell lines treated with IFN-β undergo a specific slower progression through the S-phase that seems to trigger apoptotic cellular death. In combination treatment RA potentiates IFN-β effect in SCC ME-180 but not in SiHa cell line, partially resistant to its antiproliferative action. RA added as single agent differently affects cell proliferation by inducing a slight G_1 accumulation. The IFN-β-induced S-phase lengthening parallels the increased expression of PML, a nuclear phosphoprotein specifically up-regulated at transcriptional level by IFN, whose overexpression induces cell growth inhibition and tumor suppression. We report that PML up-regulation may account for the alteration of cell cycle progression induced by IFN-β in SCC, by infecting cells with PML-PINCO recombinant retrovirus carrying the PML-3 cDNA under the control of the 5'LTR. In fact PML overexpression reproduces the IFN-β–induced S-phase lengthening. This alteration is accompanied by apoptosis induction. These findings provide important insight into the mechanism of tumor suppressing function of PML and could allow PML to be included in the pathways responsible for IFN-induced cell growth suppression.

26015

DEREGULATION OF NOTCH2 SIGNALING LEADS TO THE OVEREXPRESSION OF CD23 IN B-CLL.

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The high expression of the B-cell activation marker CD23 is one of the major characteristics of B-cell chronic lymphocytic leukemia (B-CLL). Its cleavage product, sCD23, has the profile of a multi functional cytokine and is considered as an autocrine growth factor in mature B-cells. To elucidate the mechanisms leading to the overexpression of CD23 in B-CLL, we analyzed the CD23a proximal promoter for sequence specific DNA – protein interactions. By electrophoretic mobility shift assays, we identified a transcription factor complex (C1) which binds sequence specific to one known and four newly identified putative CBF1 consensus sequences in the *CD23a* proximal promoter. The significance of this complex was highlighted by the fact, that in all B-cell samples the intensity of C1 correlates with their respective levels of CD23 expression. Furthermore, using EBV infected BL41 cells as a positive control for CBF1 mediated CD23 expression, C1 was found to be EBV inducible. Since CBF1 is the nuclear target of Notch signaling, we investigated the expression pattern of different members of the *Notch* gene family by RT-PCR and found that *Notch2* is overexpressed in B-CLL cells. This indicates, that deregulation of Notch signaling, which is known to lock cells into an immature state of differentiation and to inhibit apoptosis, might account for the overexpression of CD23 in this malignant B-cell type.

26026

Increased Levels of Vascular Endothelial Growth Factor (VEGF) in Cancer Patient Sera and Plasma versus Healthy Apparently Normal Sera and Plasma Sample Levels as Measured by a Novel ELISA for Human VEGF.

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Vascular Endothelial Growth Factor (VEGF) is a potent vascular endothelial cell mitogen that also functions as an angiogenic/vascular permeability factor. Production of new blood vessels in neoplasia is initiated via the activation of an angiogenic switch from cancer cells during the premalignant stages of tumor development. Elevated levels of VEGF expression in solid tumors are proposed to support growth via increased tumor neovascularization. Following this premise, we anticipated increased levels of VEGF in sera and plasma samples from cancer patients versus normal control levels. Using a unique enzyme linked immunoassay (ELISA) developed to specifically detect human VEGF (121 and 165kD isoforms), we measured the levels of VEGF in matched sera and EDTA plasma samples from cancer patients and apparently healthy, normal individuals. The ELISA has a dynamic range of 15.6 to 1000 pg/ml and a lower limit of detection of <5 pg/ml. Normal human sera or plasma VEGF levels were undetectable. Recovery of either recombinant or natural human VEGF spiked sera and plasmas ranged from 80-110%. There is no cross reactivity of the VEGF ELISA to Placental Growth Factor (PlGF), a related molecule of the same gene family. However, the VEGF/PlGF heterodimer does read in the ELISA at ~20-25% of mass. Samples from cancer patients of the breast, colon, lung, ovary and prostrate read ~10-fold higher(100-400 pg/ml vs. 10-40pg/ml) of VEGF than from comparative normal, healthy individual samples. Other cytokines from several species also do not cross-react or interfere with the assay. All VEGF levels reported were completely neutralized when incubated with a separate rabbit polyclonal anti-human VEGF IgG. These results demonstrate the Endogen human VEGF ELISA to be a useful tool for the quantitative analysis of VEGF.

26030

HUMAN PANCREATIC TUMOR CELLS EXPRESS MULTIPLE CYTOKINES GENES

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Cytokines are known to be involved in the regulation of a variety of immune responses. Recently, tumor cells have been shown spontaneously to secrete cytokines that downregulate or paralyze the immune system of the host and that hamper cytokine-based therapeutic intervention.
To investigate whether pancreatic carcinoma cells can be a potential source for regolatory cytokines, we analyzed the multiple cytokine gene expression in five pancreatic carcinoma cell lines and in normal pancreas using RT-PCR
Our results demonstrate that all the cell lines under investigation express mRNA for Interleukin (IL)-6, IL-8, IL-11, IL-14, IL-15, IL-16, IL-18, Transforming Growth Factor (TGF)-β1, TGF-β2 and TGF-β3; four lines out of five express IL-5, IL-7 and Granulocyte Macrophage Colony Stimulating Factor (GM-CSF); two out of five IL-1β, IL-10 and IL-12 p40. All lines express low transcript levels for M-CSF and IL-13, and none for IL-2, IL-3, IL-4, IL-9, IL-17 and Interferon (IFN)-γ. On the contrary, we found in normal pancreas tissue a high expression for IL-1β, IL-5, IL-6, IL-8, IL-15, IL-16, IL-18, TGF-β1, TGF-β2 and TGF-β3; low levels for IL-7, IL-10, IL-13, IL-14, IFN-γ and M-CSF; no IL-2, IL-3, IL-4, IL-9, IL-11, IL-12, IL-17 and GM-CSF.
These findings demonstrate that pancreas cells can be an important pro- and anti-inflammatory cytokines source. Modifications of the cytokine pattern expression occurring in pancreatic tumor cells may have important local and systemic functional consequences on immune system.

26024

Potential Roles of Monocyte Chemoattractant Protein (MCP)-1 Expressed in Draining Lymph Nodes, in Interleukin (IL)-4-mediated Tumor Rejection

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Wild-type but not IFN-γ-deficient BALB/c mice rejected a mouse adenocarcinoma, colon 26 transfected with the IL-4 gene, with concomitant generation of specific cytotoxic T cells in the draining lymph node. Because accumulating evidence indicates that chemokines have pivotal roles in the migration of macrophages and dendritic cells into lymph nodes, we next explored chemokine and chemokine receptor gene expression in the draining lymph nodes of mice inoculated with either control or IL-4-producing colon 26 cells. Among chemokine genes examined, monocyte chemoattractant protein (MCP)-1, macrophage-derived chemokine (MDC), and liver and activation-regulated chemokine (LARC) gene expression was selectively enhanced in the draining lymph nodes of mice injected with IL-4-producing cells, compared with control cell-injected mice. Moreover, MCP-1 and MDC gene expression was decreased in the draining lymph nodes of IFN-γ-deficient mice injected with IL-4-producing cells, compared with wild-type BALB/c mice. Concomitantly, the gene expression of the receptor for MCP-1, CCR2, was enhanced in the draining lymph nodes of wild type but not IFN-γ-deficient mice injected with IL-4-producing cells. Finally, the administration of neutralizing anti-mouse MCP-1 antibodies retarded IL-4-mediated tumor rejection in wild-type BALB/c mice. Collectively, these results suggest that in this IL-4-mediated tumor rejection model, the expression of CC chemokines, particularly MCP-1, is dependent on endogenously produced IFN-γ and that the chemokines have pivotal roles in IL-4-mediated tumor rejection, probably by recruiting antigen-presenting cells into the draining lymph nodes.

26022

THE ANTIVIRAL AND ANTI-GROWTH ACTIVITIES OF INTERFERON-ALPHA ARE BLOCKED BY THE PAPILLOMAVIRUS E7 PROTEIN.

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Interferon's mediate their antiviral and antigrowth effects via the activation of the JAK-STAT signaling pathway. However, *in vitro* studies show human papillomavirus (HPV)-infected cell lines appear to be resistant to the effects of Interferon (IFN)-alpha. Furthermore, international, multicenter, randomised trials have indicated there is little long term benefit to IFN-alpha treatment. One explanation for these results is the HPV is able to avoid the antiviral effects of IFN-alpha. We have investigated the role E7 plays in the resistance of HPV to IFN-alpha. We have developed a series of HPV 16 E7-expressing lines in the fibroblast cell line, 2fTGH. 2fTGH cells are dependent upon IFN-alpha for growth when selected in HAT medium. The presence of E7 resulted in the loss of IFN-alpha-dependent cell growth (p=0.0009). There was some evidence of a dose-response with lines that expressed high levels of E7 (as determined by Taqman assay) not able to grow in response to IFN-alpha while cell lines with low levels of E7 grew at close to normal rates. Similar results were observed in HaCaT epithelial cells. The antiviral effects of IFN-alpha are also lost when E7 was present. Band shift assays indicate the formation of the interferon-stimulated-gene-factor 3 (ISGF-3) transcription complex is lost in E7-expressing cells and the p48 protein is unable to migrate to the nucleus in response to IFN-alpha. Direct interaction between p48 and E7 in demonstrated. Finally we compare type 16 E7 with E7 from types-6b and -1 to see if this phenotype is associated with oncogenic potential.

26020

CHARACTERIZATION OF LIGAND INDUCED IFN-α RECEPTOR DOWN-REGULATION BY A FLOW CYTOMETRIC ASSAY

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IFN-α is extensively used in the treatment of wide range of disorders including cancer and viral disease. However, much remains to be done to optimize the therapeutic protocols with IFN-α. In addition to the studies required for the evaluation of dosage and frequency of administration, it is of great interest to establish criteria for the selection of patients likely to respond to IFN-α treatment. An early and well-documented event following IFN-α receptor interaction is ligand-induced receptor down-regulation. The decrease in the IFN-α binding sites measured by using radio-labeled IFN-α^{125} I, has been suggested as a response marker of IFN-α treatment (Billard et al., 1986, Blood, 67:821 ; Bartsch et al., 1989, Int. J. Cancer, 43:235 ; Billard et al., 1991, Leukemia Res. 15:121). By using two monoclonal antibodies against IFNAR1, one antagonizing the binding of IFN-α (64G12) and one non-antagonizing (34F10), we have investigated the involvement of the IFNAR1 chain in the IFN-α induced loss of binding sites in tumoral and in normal cells from healthy individuals. Our results showed a close correlation between the down-regulation of IFNAR1 and the loss of the IFN-α binding sites. This correlation has been obtained by using different assays : binding assays with radio-labeled IFN-α, flow cytometry with 64G12 and 34F10, biochemical characterization of the IFN-α binding complex and IFNAR1. In this study we also report a relationship between the intensity of the receptor down-regulation and gene activation (ISRE/luciferase activity) by using a tyrosine phosphatase inhibitor. In conclusion, our data shows that IFN-α receptor down-regulation is correlated with IFN-α activity and establishes a new assay based on the detection of IFNAR1 down-regulation by flow cytometry. This non radioactive assay is simple and could be used in clinical routines to follow patients treated with IFN-α.

26019

Reduced malignancy patterns of metastatic breast cancer cells engineered to overexpress IL-1α.

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Vaccine preparations against cancer cell, that are based on methods of gene transfer of cytokines, are aimed to regulate the cytokine network in proximity of the tumors and thus enhance the development of local immunity against the malignant cells. As an experimental model for breast cancer, we have used the TSA mammary adenocarcinoma of BALB/c mice, which spontaneously metastases to the lungs following its subcutaneous inoculation. Here, we report on the immunotherapeutic efficiency of IL-1α in cytokine gene-transfer approaches of metastatic tumors. The cytokine IL-1α is a good candidate for immunotherapy because it is a pleiotropic cytokine which activates all arms of the immune system, however, its immunotherapeutic potential has not been appropriately studied. IL-1α is mainly active as a cytosolic precursor or in its membrane-associated form, however, it is not secreted. TSA cells were transfected with the cDNA of the precursor of murine IL-1α. We have used two clones of transfectants: TSA-8 that produce 200-300 pg/10^6 cells/24 hrs and TSA-22 that produce 30 pg/10^6 cells/24 hrs. When injected intrafootpad into mice, wild type cells ($3x10^4$ /mouse), all mice developed tumors within 17 days, whereas TSA-8 did not induce tumor growth and TSA-22 induced tumors in 80-90% of the injected mice and larger numbers of metastases in lungs were observed, compared to TSA wild-type. Another group of mice, that had been injected i.f.p with wild type cells, the tumor was amputated and then mice were treated i.p with TSA-8 (treated with Mitomycin-c), longer survival was observed, as compared to amputated and non-treated mice. Preliminary results indicate that T cell mediated immune responses as well as NK cells reactive against the malignant cells mediate reduced metastasis of IL-1α-transfected TSA-8 cells. Thus, IL-1α in its membrane-associated form may serve as an adhesion molecule, promoting cell-to-cell interactions between immune effector cells and the tumor cells, and as a focused adjuvant, thus exerting potent anti-tumor activity at very low levels of expression, far below those which are toxic to host. Further studies are aimed to evaluate the anti-metastatic potential of IL-1α-based tumor cell vaccines.

26018

CAN A SMALL DOSE OF INTERFERON BE EFFECTIVE?
·CLINICAL EVALUATION OF THE IFN·ALPHA ACTIVATED NK CELL THERAPY (IFNANK) FOR CANCER PATIENTS·

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【Aim】 A large quantity of interferon (IFN) is clinically administered for patients with malignancy or chronic viral hepatitis that is sometimes accompanied with various side effects. Since 1994, Kishida et al invented the new immunotherapy that required a small dose of IFN termed "IFN Activated NK cell therapy (IFNANK)". The effects were clinically evaluated in 78 patients treated with IFNANK more than 6 times. 【Methods】 Auto·lymphocytes (about 5×10^9 cells) collected via leukopheresis was subsequently re·infused into the patient intravenously after short term treatment with a small quantity (10^5 units) but high concentration (500 u /ml) of IFN·alpha in vitro. Most subjects(87%) were in advanced stages. 【Results】 None of subjects suffered any significant side effects. Clinically succesful cases were 40 cases including 18 without relapse, 1 CR, 12 PR and 9 long NC. The 3 year survival of these patients was 28% in stage Ⅳ. Tests of immunological parameters revealed increase of 2·5AS in most subjects, and its amplification rates were significantly larger in succesful cases than in poor ones. NK activities measured by original methods using flow cytometry (by Kishi, Fujita et al) were demonstrated remarkable elevations in collected lymphocytes following treatment of IFN, especially in subjects with good clinical courses. 【Conclusion】 The in vitro activation in IFNANK requires a small dose of IFN for the optimal conditions. Clinical studies showed its' usefulness. And it indicates the wide possibility of clinical applications of other cytokines.

26016

VACCINATION WITH IRRADIATED TUMOR CELLS FOLLOWED BY SUPERANTIGEN ADMINISTRATION ELICITS A POTENT AND PROTECTIVE ANTI-TUMOR IMMUNE RESPONSE IN C57Bl/6.

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B16F10 melanoma is a tumor derived from C57Bl/6 mice, which has been found to be poorly immunogenic and highly aggressive. We have been shown that vaccination of mice with irradiated B16F10 cells followed by treatment with a combination of the superantigens staphylococcal enterotoxin A and B (SEA/SEB) leads to potent and specific protection against subsequent challenge with viable B16F10 cells. Further, mice surviving over 150 days remained tumor-free following rechallenge with viable B16F10 cells, suggesting the development of strong immunological memory. Superantigen treatment of vaccinated mice resulted in increased $CD4^+$ and $CD8^+$ T cells, induction of CTL activity, and $CD4^+$ T cell induction of the cytokine $IFN\gamma$. $IFN\gamma$ was shown to directly inhibit melanoma tumor cell replication via the induction of the tumor suppressor gene product $p21^{WAF1/CIP1}$. All of these events probably play a role in the significant enhancement of an otherwise ineffective anti-tumor immune response. We therefore conclude that superantigens are potent adjuvants for enhancement of tumor immunity.

26017

The level of Cytokines in the blood serum of the patiens with multiple melanoma.

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Currently at the Russian Cancer Researsh center AMS it was registrated positive clinical effect of the therapy of malignant melanoma (1pt), colon carcinoma (1pt) and renal carcinoma (1pt) with interkeukin-1β (IL-1β). In two first cases IL-1β has been used as single agent, and in third one - together with chemotherapy. Now we investigated the level of cytokines in the blood serum of 39 multiple melanoma patients, treated with polychemotherapy alone (31 pts) or in the complex with human recombinant IL-1β (Betaleukin) and α-2 interferon - Roferon A (Rof A) (8 pts). It has been tested the concentration of IL-2, IL-6 TNF-α and IL-2 receptor (IL-2R) in blood serum of the patients by ELISA-method, using kits, produced by Diaclon or Boehringer Mannheim Co. Non of the patients had IL-2 in the serum. 26 pts had no IL-6 and only 13 had 20 - 120 pg/ml of IL-6. The most of patients with positive clinical effect (complet or partial remission or stabilization of desease) had no or only very low level of TNF-α in their serum, but progressor patients had higher concentration of this cytokine. Most of patients showed very high concentration of soluble IL-2R, but 3 patients during partial remission had very low level of soluble IL-R. This data need in integrated statistic analysis and it is necessary to investigate the expression of TNF-α receptor on the melanoma cells of successfully treated patients.

26027

CYTOKINE EXPRESSION IN NORMAL AND MALIGNANT PROSTATE EPITHELIUM. FOCUS ON IL-18 SYSTEM

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In addition to regulating proliferation and differentiation in cancer cells, cytokines also affect tumor host interaction by controlling immune and inflammatory responses. In the present study cytokine expression was determined in prostate cancer cell lines. Gene expression of 24 cytokines in normal prostate epithelium, androgen-dependent (AD) and androgen-independent (AI) prostate cancer cell lines were analyzed utilizing TaqMan real-time quantitative RT-PCR assay. In a primary culture of benign epithelial cells, PrEC, IL-1α, IL-1β, GM-CSF and TGFβ were highly expressed but IL-6, IL-8, IL-18, TNFα and M-CSF were also detected. Expression of IL-2, IL-3, IL-4, IL-5, IL-10, IL-12, IL-13, IL-15, IL-17, LT-β, TNFβ and IFNγ was low or absent. In contrast, proinflammatory cytokine gene expression was reduced in malignant prostate cells. In the AI cell line DU 145 the proinflammatory cytokines IL-1α, IL-1β, TNFα and GM-CSF were significantly low (TNFα was 1000 fold less and IL-1β was 10000 fold less than in PrEC). Nevertheless, levels of IL-6, IL-8, IL-18 and TGFβ remained unchanged. In the AD cell line LNCap, global suppression of cytokine genes was observed. mRNA levels of all 24 cytokines were below the assay sensitivity, whereas amplification of 18S mRNA was the same as in normal prostate epithelium. Because IL-18 is a regulator of Th1 responses and immune defense against cancer, expression and production of IL-18 and IL-18 binding protein (IL-18BP) in normal and malignant prostate cell lines was studied. IL-18 gene expression and protein production were detected in normal epithelium PrEC and in six AI cancer cell lines: PPC-1, PC-3, TSU, ALVA, DU 145, JCA. In contrast, AD cancer cell lines LNCap and LAPC4 did not express either IL-18 protein or mRNA. In general, expression of IL-18BP corresponded to IL-18 expression; however, in the AD line LAPC4, not expressing IL-18, IL-18BP mRNA was present, whereas in AI lines expressing IL-18, IL-18BP mRNA was low. These data support our hypothesis that in prostate epithelium the process of malignancy leads to suppression of proinflammatory cytokine production thereby inhibiting local immune and inflammatory responses.

Chemokines, HIV and vaccine

27004

Chemokines, chemokine receptors and HIV or SIV vaccination

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The primary function of the β-chemokines, RANTES, MIP-1α and MIP-1β is to attract macrophages, immature dendritic cells, T and B cells expressing CCR5, CCR1 or CCR3. This chemokine-receptor interaction mobilizes the essential immune cells which in the presence of an antigen exert an adjuvant effect on antibody and T cell responses. HSP70 has been identified as a common agent present in most microorganisms that stimulates the 3 β-chemokines which then engage CCR5 or the other receptors in what appears to be an innate adjuvant mechanism. The HSP70 however, has a groove which binds peptides that may elicit an adaptive immune response specific to the peptide. Indeed, ATP-treated HSP70 can be loaded in vitro with peptides that will elicit a cascade of innate, followed by adaptive immunity. The general principle of innate immunity elicited by HSP70-peptide complexes driving adaptive immunity is of special significance in HIV vaccination, as HIV has subverted CCR5 as a primary coreceptor in cellular transmission of the virus. Thus, HSP70-HIV peptide complexes will induce β-chemokines that will attract the repertoire of immune cells and induce a cognate response to the peptides, whilst at the same time inhibiting HIV infection by blocking and downmodulating CCR5. The HIV non-cognate mechanism can be further enhanced by inducing antibodies to CCR5, as its extracellular domains are immunogenic. The novel strategy utilising HSP70-peptide complexes generates cognate responses to the HIV peptides and innate β-chemokine responses which further inhibit HIV transmission by blocking the CCR5 receptors.

27002

Inhibition of HIV Infection by the Cytokine Midkine

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We have recently reported that the growth factor midkine (MK), implicated in differentiation and development, binds to the cell-surface expressed nucleolin and results in the inhibition of HIV infection in different cell lines as well as in peripheral blood T-lymphocytes. MK binds cells specifically since this binding is inhibited by unlabeled MK but not by other related growth factors. MK-pretreated cells resist HIV infection by the failure of HIV particles to become attached to cells via a mechanism independent of CD4. Here we show that MK mRNA is detectable systematically in adult peripheral blood lymphocytes from healthy donors. Upon activation of T-lymphocytes with PHA or with anti-CD3/anti-CD28 antibodies, the expression of MK is increased significantly in a transient manner. MK expression is also induced upon stimulation through the CD28 receptor alone or treatment with IL-2 or IFN-γ. In view of its enhanced expression in response to physiological agents along with its various growth factor effects, MK should be considered as a cytokine with a potential function in homeostasis. As examples for the potential control of HIV infection by MK, we then show that $CD4^+$ T-cell clones expressing constitutively MK resist HIV infection via inhibition of HIV particle attachment to cells. Furthermore, when $CD4^+$ cells are cocultured with MK producing CD4-negative cell clones, they become resistant to HIV infection. Taken together, our results suggest that MK is a cytokine that could regulate HIV infection in an autocrine or paracrine manner and thus be implicated in HIV pathogenesis.

27006

β CHEMOKINE EXPRESSION DOWNREGULATED BY HIV-1 THROUGH A TGF-β-DEPENDENT MECHANISM

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The β chemokines MIP-1α, MIP-1β, and RANTES are potent inhibitors of HIV-1 entry into T cells and are believed to play a role in host-protective anti-viral responses. The principal sources of β chemokines are macrophages and T lymphocytes, and multiple stimuli have been shown to elicit β chemokine release from these two cell types. We have demonstrated that co-incubation of macrophages and primed T cells, neither of which release β chemokines when cultured alone, leads to the sustained release of MIP-1α and MIP-1β in a cell-cell contact-dependent process. In this co-culture system, MIP-1α and MIP-1β expression is upregulated in both cell types. Upregulation of β chemokines in the macrophage compartment is dependent upon the expression of CD40 on macrophages and the expression of CD40L on the primed T cells. Macrophages infected with HIV-1 prior to co-culture with lymphocytes released markedly less MIP-1α and MIP-1β than uninfected macrophages. This effect was observed with both syncytium-inducing and non-syncytium-inducing HIV-1 strains, and was blocked by the addition of antibodies to TGF-β. Inoculation of purified macrophage cultures with HIV-1 induced the release of bioactive TGF-β, and addition of exogenous TGF-β to co-cultures down-regulated CD40 expression on macrophages and subsequent release of β chemokines. In ex vivo studies utilizing cells isolated from HIV-infected individuals, co-culture chemokine induction appeared to be defective. Factors contributing to lower β chemokine release were decreased CD4 counts, failure of CD4 T cells to effectively upregulate CD40L upon activation, and decreased resting and inducible CD40 expression on macrophages. These results reveal molecular mechanisms that allow HIV-1 to avoid the anti-viral activity of β chemokines in vivo.

27005

THE STRENGTH OF CD28 COSTIMULATION DETERMINES ENHANCEMENT OR INHIBITION OF R5 HIV REPLICATION.

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We had previously reported that CD4 T cells infected *in vitro* and stimulated with sepharose beads coated with anti-CD3 and anti-CD28 do not support CCR5-dependent (R5) HIV replication, whereas high levels of viral replication could be induced by cross-linking CD3 and CD28 with soluble anti-CD3 and cells that express B7.1 and Fc receptors. Suppression or enhancement of HIV replication correlates with the ability of anti-CD3/CD28 beads (and not of anti-CD3/B7.1) to downregulate CCR5 expression and induce the secretion of high amounts of β-chemokines.

We show here that suppression of R5 HIV replication is not specific to bead-bound anti-CD3/CD28, and not necessarily correlates with β-chemokine secretion, but depends on the strength of CD3/CD28 stimulation. Specifically, by increasing the strength of CD3/B7.1 stimulation we were able to achieve the same suppression of HIV replication observed with anti-CD3/CD28 beads, in a condition that induced minimal levels of β-chemokines. Moreover, at low CD3/CD28 stimulation levels, CD4 T cells efficiently supported R5 HIV replication, independently of the mode of cross-linking CD3 and CD28.

Thus, cellular activation is required to induce HIV replication, but full activation suppresses R5 HIV, through a β-chemokine independent mechanism.

27003

ELEVATED EXPRESSION OF β-CHEMOKINE RECEPTORS: RELEVANCE TO THE INCREASED SUSCEPTIBILITY TO HIV INFECTION IN AFRICA?

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Background: We have previously described increased susceptibility of PBMC obtained from Ethiopian immigrants (ETH) to Israel for HIV infection[1], which was ascribed to increased CCR5 expression on monocytes and lymphocytes[2]. Here we studied the role of environmental *vs* constitutive factors in this phenomenon.
Methods: CCR5 and CXCR4 expression on CD4+ cells and several T-cell activation markers were determined by triple-colour flow cytometry in 26 ETH upon arrival (new-ETH), 22 ETH five years after immigration (old-ETH) and 20 non-ETH Israelis.
Results: While the new-ETH were highly immune activated, the old-ETH and the non-ETH were not. However, the proportion and number of CCR5+CD4+ cells and the number of CCR5 molecules/CD4+ cell, in both HLA-DR+ and HLA-DR- compartments, were higher in the ETH groups as compared to non-ETH group ($p<0.0001$). Similarly, in both ETH groups, the number of CXCR4 molecules/CD4+ cell, in both HLA-DR+ and HLA-DR- compartments, was also higher ($p<0.01$). CCR5 and CXCR4 expression correlated with immune activation (proportion of HLA-DR+CD4+ cells) only in the non-ETH control group.
Conclusions: The elevated CCR5 and CXCR4 expression found in ETH upon arrival to Israel does not decrease even after their immune profile returns to normal. Thus, such increased HIV co-receptors expression in ETH may reflect a constitutive unique genetic trait, which probably contributes to the increased susceptibility to HIV infection observed in them[1] and to the rapid spread of AIDS in Africa.

1. AIDS 1998; 12:1731.
2. J Hum Virol 1999; 2:283.

27001

Deregulation of the Expression of the Fractalkine/Fractalkine receptor complex related to viral replication in HIV-1-infected patients

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Fractalkine is the only member of the CX3C chemokine family. Polymorphism of the fractalkine receptor gene influences the prognosis of HIV infection. To gain insight into the mechanism of this effect on prognosis, expression of fractalkine and of its receptor was analyzed in HIV-infected patients. In contrast to HIV-uninfected individuals, a large number of cells expressed fractalkine in interfollicular areas of lymph nodes from HIV-infected patients. Dendritic cells were involved in this increased expression. A large number of cells in the duodenum from HIV-infected patients, but not from controls, also produced fractalkine. The fraction of CD45RO+ and CD45RO- T helper cells expressing the fractalkine receptor was higher in HIV-infected patients than in healthy individuals, and these cells were abnormally sensitive to fractalkine stimulation. This increased response correlated with HIV viremia, and it returned to normal levels in patients successfully treated with antiretroviral drugs. The increased expression of the fractalkine/fractalkine receptor complex in HIV infection may affect adhesion and migration of T helper lymphocytes and their interaction with dendritic cells. Thus, it may influence the equilibrium between depletion and renewal of the Th compartment.

27009

ACTIVATION OF THE FORMYL PEPTIDE RECEPTORS PHOSPHORYLATES AND DESENSITIZES THE CHEMOKINE RECEPTOR CCR5 AND INHIBITS HIV-1 INFECTION

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Interactions between cell surface receptors are important regulatory elements in the host response to infections. Here we show that activation by a classic chemotactic factor, the bacterial chemotactic peptide N-formyl-methionyl-leucylphenyl-alanine (fMLF), rapidly induced a PKC-mediated serine phosphorylation and down-regulation of the chemokine receptor CCR5, which serves as a major HIV-1 coreceptor. Interaction of fMLF with its receptor, FPR, resulted in significant attenuation of cell responses to CCR5 ligands, and also inhibits HIV-1 envelope glycoprotein mediated fusion and infection of cells expressing CD4, CCR5 and FPR. Similar desensitizing effect on CCR5 was also observed following activation of a low affinity fMLF receptor, FPRL1. The finding that the expression and function of CCR5 can be regulated by peptides that use unrelated receptors may provide a novel approach to the design of antiinflamatory and antiretroviral agents.

27007

ENTRY OF HIV-1 NEF PROTEIN IN HUMAN MONOCYTE/MACROPHAGE PRIMARY CELLS INDUCES THE ACTIVATION OF THE SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION STAT 1.

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Nef is a cytoplasmic multifunctional viral protein expressed in abundance during the early phase of HIV replication, that is able to selectively downregulate cell surface expression of the CD4 receptor and of the MHC-class I molecules, to up-regulate the expression of Fas ligand and to interact with specific cellular signalling protein such as lck, hck and Vav. To investigate if Nef was able to perturb the JAK/STAT cytokine signal transduction pathway, we have used as model system human monocyte derived macrophage (MDM) from healthy donors treated with recombinant (rec) Nef of the NL4-3 strain. Through confocal microscope analysis, we first demonstrated that MDM efficiently internalize Nef that disposes in an intracytoplasmic pattern highly reminescent of that reported in cells endogenously expressing Nef. Then we analysed STAT 1 activation by western blot analysis with anti phospho-Y-STAT1 or anti-STAT1 antibodies. Kinetic studies showed a strong activation signal after 2 hrs of cell treatment with rec Nef followed by the increase of STAT 1 expression. STAT 1 activation was further confirmed via band shift experiment using both the GAS element of the IRF-1 promoter and the SIE element of the c-fos promoter. Immunodepletion of Nef from the Nef containing medium used to treat MDM failed to induce STAT 1 activation, demonstrating the specificity of the effect. STAT 1 serine phosphorylation was also increased suggesting that Nef treatment induces both the formation of the GAF DNA binding factor and its transactivating function on gene transcription. Accordingly the expression of the transcription factor IRF-1, that is transcriptionally regulated by STAT 1, is also increased. Nef induced activation of STAT 1 could influence both the efficiency of viral expression and the macrophage response to infection. Further studies are in progress to evaluate if STAT 1 activation by Nef is mediated via the secretion of activating factor released by the Nef treated MDM.

27008

PREFERENTIAL ENTRY OF M-TROPIC HIV IN EPITHELIAL CELLS DUE TO OPPOSITE EFFECTS OF INTERFERONγ ON CXCR4 AND CCR5 EXPRESSION

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Cells of the epithelial type are the first cells encountered by HIV during sexual transmission and breastfeeding; they may replicate HIV both *in vivo* and *in vitro* and spread internalised virus by transcytosis. We studied *in vitro* the modulation of CCR5 and CXCR4 HIV co-receptors as well as the entry of T- or M-tropic HIVs in epithelial cells of cervical, colonic and bladder origin, and in endothelial cells. We used IFNgamma since it is a major interplayer of immune responses, and it has been reported to have suppressive/enhancing effects on HIV. The expression of CCR5 and CXCR4 co-receptors was evaluated at RNA and protein level after overnight incubation with/without IFNgamma, in parallel to binding and replication of T-tropic or M-tropic HIV-1 strains, by qualitative and semiquantitative RT-PCR and PCR, Western blotting, p24 antigen, and infectivity assays. The results indicate that in cells exposed to IFNgamma CXCR4 expression is reduced while that of CCR5 is stimulated, dose-dependently, in terms of both RNA and protein accumulation. Interferon-treated and control cells were infected with T-tropic or M-tropic HIV strains and virus binding and entry were determined. Evaluation of p24 antigens and PCR analysis specific for HIV *gag* proviral DNA showed reduced T-tropic and increased M-tropic virus binding and provirus accumulation. Virus yields varied accordingly. The conclusion was that co-receptor modulation occurs also at epithelial level. Inflammatory stimuli may provide selective advantages for binding of HIV strains with different tropism. In facts differential regulation of CCR5 and CXCR4 IFNgamma helps explaining the selection of M-tropic strains at mucosal level. This may occur also at endothelial level.

Interferons and cytokines in infectious disease I

28008

cDNA CLONING OF BIOLOGICALLY ACTIVE CHICKEN INTERLEUKIN-18

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We identified a candidate chicken interleukin-18 (ChIL-18) cDNA in a bursal EST data base that codes for a 198-amino acid protein which shows 28 to 34% sequence identity to IL-18 of mammals. Like its mammalian counterparts, ChIL-18 appears to be synthesized as a precursor molecule that is processed by a caspase 1-like protease to yield a mature protein of 18.6 kDa. A histidine-tagged variant of this molecule was expressed in E. coli and purified by nickel agarose affinity chromatography. The purified material induced the synthesis of IFN-γ in suspensions of explanted chicken spleen cells, indicating that recombinant ChIL-18 is biologically active.

28004

PHENOTYPE OF IFN-ß NULL-MUTANT MICE: IMMUNE STATUS

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To examine the *in vivo* role(s) of IFN-ß in normal development and the host immune response to infections and tumorigenesis, mice with a null mutation in the IFN-ß gene were generated. There are no gross signs of abnormal fetal development or overt phenotypical changes in adult IFN-ß$^{-/-}$ mice. However, IFN-ß$^{-/-}$ mice are highly susceptible to viral infection, exhibiting a dramatic reduction in virus inducibility of IFN-αs. We provide evidence for diminished virus-inducible IRF-7 expression in IFN-ß$^{-/-}$ target cells, directly associated with this restricted IFN-α inducibility. These data indicate a pivotal role for IFN-ß in the innate immune response to virus infection.
Furthermore, IFN-ß$^{-/-}$ mice display abnormalities in T, B and myeloid lineages, thereby affecting the adaptive immune response. Specifically, there is a 3-fold increase in cellularity in the thymus of adult IFN-ß$^{-/-}$ mice compared with IFN-ß$^{+/+}$ mice, distinguished as elevated levels of double positive CD4/CD8 T cells. By contrast, there is a decreased cellularity in the bone marrow of IFN-ß$^{-/-}$ mice, associated with a reduction in IgM^{+} B cells. The implications are that in both the thymus and bone marrow IFN-ß exerts a positive effect on lymphocyte maturation. We observe no changes in the proportion of hematopoietic cells in the spleens of adult IFN-ß$^{-/-}$ mice. However, elevated levels of myeloid lineage cells were detected in peripheral blood from adult IFN-ß$^{-/-}$ mice, identified as a 2-fold increase in Mac-1 and Gr-1 positive staining cells. Viewed altogether, these results define an important role for IFN-ß in normal hematopoietic development. We conclude that IFN-ß protects the normal adult host from virus infection by directly regulating intact innate and adaptive immune responses.

28006

PARAMYXOVIRUS SV5 IS PATHOGENIC IN MICE LACKING STAT 1

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SV5 is a well-studied member of the paramyoxvirdae family. Although it is non-pathogenic for humans and many other animals, it has been used as a model for the study of paramyxovirus genetics and biochemistry. The development of a recombinant infectious clone of SV5 has allowed virologists to generate viral mutants which can then be used to study viral gene function. In recently published work, a recombinant SV5 strain lacking the SH gene was generated and compared to parental wild type virus in tissue culture studies. The SH-deleted strain did not in fact differ from wild type in its ability to to replicate in cultured cells. The contribution of SH to pathogenesis could not be determined due to the non-pathogenicity ofSV5 in any convenient animal model. To determine whether SV5 could infect IFN non-responsive mice, Stat1-deficient BALB/c mice and wild type controls were inoculated intranasally with increasing doses of virus. In this study we found that Stat1 -/- mice were indeed susceptible to SV5 (LD_{50}=10^{5} pfu) while wild type animals infected with 10^{6} pfu remained asymptomatic. Using the Stat1-/- mouse model of SV5 infection, we then examined the effect of SH gene deletion on pathogenesis. The SH-deleted virus, which replicates as well as wild type virus in cultured cells, is in fact less pathogenic than wild type SV5 in Stat1-/- mice (LD_{50} greater than 10^{6} pfu). This finding demonstrates the potential utility of Stat1-/- animals to study the biology of viruses unable to infect wild type mice.

28002

CIRCADIAN RELATIONSHIP BETWEEN CORTISOL AND MACROPHAGE INHIBITORY FACTOR (MIF): EVIDENCE FOR A NEURO-ENDOCRINE INTERACTION

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MIF is expressed by activated immune cells and has the unique property of counter-regulating the anti-inflammatory and immunosuppressive effects of glucocorticoids. MIF also is secreted by the anterior pituitary gland in response to stress. Notably, plasma MIF levels have been shown to increase after glucocorticoid administration in experimental murine models, suggesting that MIF acts together with glucocorticoids to effect a balance between pro- and anti-inflammatory effects. We wished to determine the relationship between circulating cortisol and MIF levels in human subjects so as to better understand MIF's dual role as a pituitary-derived hormone and immune cell-derived cytokine. Ten healthy volunteers had blood drawn each hour for 24 hours for measurement of plasma cortisol and basal and stimulated cytokine production. Plasma MIF peaked during the late morning at approximately the same time as cortisol whereas the pro-inflammatory cytokines IFNγ, TNFα, IL-1β, and IL-12 peaked in early morning when plasma cortisol levels were low. On a second occasion, subjects received a 25 mg oral dose of cortisone acetate at 9 pm. After oral cortisone, plasma MIF rose 2-8 fold whereas IFNγ, TNFα, IL-1β, IL-12 and, to a lesser extent, IL-6 and IL-10 sharply decreased. MIF was the only cytokine whose circulating level increased rather than decreased in response to cortisol. Diurnal entrainment of inflammatory cytokine expression by cortisol has been proposed to contribute to nocturnal exacerbations of disorders such as rheumatoid arthritis or asthma. The late morning peak of MIF, by antagonizing cortisol-mediated cytokine inhibition, could prolong the duration of early morning inflammation and may explain in part the beneficial effects of MIF neutralization in animal models of inflammatory disease. These first data in human subjects support a novel neuro-endocrine pathway of immune regulation that involves a dual role for MIF as a circulating hormone and a pro-inflammatory cytokine.

11001

Therapy with interferon alfa 2b and ribavirin in naïve patients with chronic hepatitis C

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Our aim was to evaluate the efficacy and safety of the treatment with recombinant interferon alfa 2b (rIFNα-2b) and ribavirin (RV) in naïve patients with chronic hepatitis C. **Methods:** We included 100 patients with detectable HCV RNA, positive anti-HCV, chronic alanine aminotransferase (ALT) elevation, biopsy proven chronic hepatitis, and compensated liver disease. They received rIFNα-2b (3MU tiw, sc) plus randomly and double blind assigned RV (1200 mg/day, orally) (group I, n=50) or identical placebo (group II, n=50), during 48 weeks. Therapy was concluded in non-responders at 24 weeks. End-of-treatment and sustained (24 weeks after the end of therapy) biochemical (normal ALT) and virological (negative HCV RNA) responses were evaluated on the basis of an intention-to-treat analysis. **Results:** Twenty eight patients (56%) from group I and 17 (34%) from group II achieved an end-of-treatment biochemical response (p=0.03). Therapy was concluded at 24 weeks in 10 non-responders (20%) from group I and 22 (44%) from group II (P=0.01). One patient (2%) from group I and 5 (10%) from group II relapsed during treatment (P=0.09). An end-of-treatment virological response was observed in 23 patients (46%) from group I and 14 (28%) from group II (P=0.06). Eighteen patients (36%) from group I and 6 (12%) from group II achieved both biochemical and sustained responses (P=0.005). The treatment was discontinued due to abandon or severe toxicity in 11 patients (22%) from group I and 6 (12%) from group II (P=0.18). **Conclusions:** The combination of RV and rIFNα-2b is the therapy of choice for naïve patients with chronic hepatitis C.

28003

A NOVEL DYNAMIC EQUATION TO REPRESENT AND STUDY VIRUS-HOST INTERACTIONS IN HCV INFECTED PATIENTS.

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In absence of reproducible cell lines systems for hepatitis C virus (HCV), mathematical models (Neumann et al., Science 1998 and Zeuzem et al., Hepatology 1998) have been proposed as surrogate methods to evaluate the in vivo effects of antivirals on the virus life cycle. The differential equations used in both models consider the immune system activity as a constant factor, so that the number of hepatocytes under immune attack is the product of such constant by the infected cells number. Hence, in a dynamic setting, any change in the equation affecting viral load would cause an unlimited increase or decrease of vireamia (unsteady state). Clearly, this behaviour is not observed in nature. To overcome the problem we represented the immune system activity as a function positively correlated to the number of infected hepatocytes, so that a negative feedback on viral load is introduced (steady state). We also assumed that cytokines and antivirals might modulate the immune system activity that is correlated to the infected cells number (relevant when the number of infected cells is high) and/or activate an immune response independent on the number of infected cells (relevant when the number of infected cells is low). To gain more information about the other parameters involved in viral dynamics, we also introduced a relationship between alanineaminotransferase (ALT) levels and the number of infected cells under immune attack. Firstly we applied our model to predict patterns of ALT and HCV viraemia during therapy after measuring such variables at given times during the first month.The model was tested in 9 chronic hepatitis C patients treated with 5-9 MU of recombinant alpha IFN tiw for 3 months (non responders, 3 patients) or 1 year (primary responders, 6 patiens). The model fitting of the first 4 weekly determinations of ALT and HCV-RNA serum levels during treatment could correctly identify those patients who reached normal ALT and negative HCV-RNA (below 1000 copies/ml by our in house RT-PCR) by the end of therapy. Furthermore, fitting ALT and HCV-RNA (Amplicor Monitor, Roche) measurments obtained in a cohort of 20 HCV patients treated for 2 months with 500 or 1000 mg daily of Mycophenolate Mofetil (another IMP dehydrogenase inhibitor as Ribavirin) and then with the combination of 3 MU IFN every other day, served for testing different modes of action for each drug. This model may provide a new tool to evaluate the effects of any new or combination drug associated to IFN in hepatitis C therapy.

28005

PREDICTIVE MARKERS OF RESPONSE TO INTERFERON α IN HEPATITIS C PATIENTS.

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Attempts to find reliable means of predicting which chronic hepatitis C patients are likely to respond to IFN treatment are currently being performed. We tried to address such issue by verifying whether the early measurement of some virus- or host-markers could be predictive of the degree of the IFN-induced decrease of the viral load and, consequently, of the clinical outcome of the therapy. Specifically the following markers have been considered: Hepatitis C virus (HCV) genotype, ALT levels, plasma and PBL-associated viral load, expression of IFN α induced proteins, level of circulating IFN. The above markers have been analyzed very .early during the therapy and specifically in a group of 30 chronic hepatitis C patients at 0, 24, 48, 72 hrs from the first IFN α injection ($3x10^6$IU) and in a restricted group of 10 patients at baseline and at 2, 4, 6, 8, 12, 16, 24, 48 hrs from the first injection of IFN α. The study is still in progress but the results so far obtained indicated that while serum IFN α concentration peaks in most of the patients (80%) at 6 hrs since the injection, the expression of MxA in PBL varies considerably on individual basis, the peak of expression being between 4 (30%) and 18 hrs (20%). A significant correlation was found between the baseline expression of MxA mRNA and baseline HCV-RNA load (r=0.923; p<0.001). An inverse correlation (r= - 0.735; p=0.04) was also found between the baseline expression of MxA mRNA and the degree of early reduction of HCV-RNA load measured at 24/48 hrs from the first injection of IFN α, the fall of HCV-RNA being significantly higher in patients with lower baseline MxA mRNA level. Furthermore this study also revealed that: in 75% of the patients examined a significant reduction (more than 0.3 Log) of HCV viral load could be recorded after 24 hrs and/or 48 hrs (range 0.36-2.54Log); the baseline viral load levels and ALT levels do not influence the level of early HCV RNA reduction measured in PBL and plasma. Importantly, the HCV reduction measured in PBL and in plasma at 48 hrs from the first injection of IFN α was correlated with the harbouring genotype and, in a preliminary analysis, with the outcome of therapy.

28019

PKR PROTECTION AGAINST INTRA-NASAL VESICULAR STOMATITIS VIRUS INFECTION IS MOUSE STRAIN DEPENDENT

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The interferon-induced anti-viral state is mediated by interferon stimulated genes that are up-regulated in concert following type I interferon induction or treatment. The precise mechanism by which one or a number of these genes protects against infection by a particular viral pathogen is well understood in only a few cases e.g. the Mx protein and influenza. The double stranded RNA dependent protein kinase PKR is thought to be a major player in anti-viral defense since so many viruses encode strategies shown to inactivate PKR. PKR-deficient mice have previously been shown to be resistant to intravenous vesicular stomatitis virus (VSV) inoculation. We have confirmed that observation, but have also demonstrated increased susceptibility of PKR-/- animals to VSV infection by the intra-nasal route. This increased sensitivity, compared to wild type animals, is on the order of 1.5 logs as measured by LD_{50}. This result parallels in vitro studies using using MEFs derived from wild type and PKR-/- animals. We have found that this difference is best appreciated using PKR-/- mice on a defined genetic background since large differences in sensitivity exist between mouse strains. While there is a 1.5 log difference in susceptibility between wild type and PKR-/- mice when compared on the 129SvEv strain background, we have found that the difference between wild type BALB/c and 129SvEv animals is on the order of 5 logs. While this result clearly demonstrates an important role for PKR in protection against VSV infection, it also underlines the importance of other host factors interacting with the interferon response.

28020

Mx PROTEINS INTERACT WITH COMPONENTS OF PML NUCLEAR BODIES

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Interferon (IFN)-induced murine Mx1 protein has antiviral activity against influenza viruses. It accumulates in (or juxtaposed to) nuclear structures of unknown function, called PML Nuclear Bodies (NBs). NBs consist of a number of proteins, some of which are inducible by type I IFN. In addition, several NB constituents are modified by the ubiquitin-related protein SUMO-1. To better understand the antiviral mechanism of Mx1 protein, we started a search for cellular proteins capable of interacting with Mx1. Using the yeast two-hybrid system, we identified a number of nuclear proteins that are candidate interaction partners of Mx1. Interestingly, three of these candidates are components of NBs, namely SP100, SUMO-1 and Ubc9. In addition, the recently described Mx-interacting serine/threonine kinase PKM (1) accumulates in NBs and colocalizes with Mx1. Moreover, Mx1 interacts with both enzymes involved in sumoylation of proteins, i.e. the SAE2 subunit of the SUMO-activating enzyme and the SUMO-conjugating enzyme Ubc9. NBs are known to contain nascent mRNA. It is conceivable that they constitute the sites where Mx1 interferes with primary transcription of influenza viruses and that sumoylation may play a role in the antiviral activity of Mx1.

(1) Trost, M. et al., J. Biol. Chem. (2000) 275:7373

28021

SENSITIVITY TO IFN PREPARATIONS AND IFN INDUCERS IN PATIENTS WITH DIFFERENT VIRAL INFECTIONS

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Now it's known that immunnotherapy of patients with viral infections plays the important role for successful treatment. We propose to determine the individual sensitivity of patients to IFN preparations and other immunomodulators for individual treatment of patients with viral diseases

250 patients with hepatitis C (HCV), 150 patients with herpes infection (HSV 2+CMV), 60 healthy volunteers were studied using 7-10 ml of their whole blood. IFN priming effect was used for testing individual sensitivity to IFN-α (Intron A, Roferon A, Wellferone, Reaferon, Leukinferon, Realdiron, Human Leukocyte Interferon), IFN-γ (Gammaferon) Ridostin, Cycloferon, Kagocel were used for induction of human blood cells for determination of sensitivity to IFN inductors.

Present investigations showed that patients with different diseases have diverse sensitivity to preparations (Table).

Table. Sensitivity to IFN preparations and IFN inducers in patients with viral infections.

Preparations	Sensitivity (per cent patients).		
	Healthy	HSV+CMV+chlamidiosis	HCV
Cycloferon	73	25	25
Ridostin	98	45	62
Kagocel	79	-	50
IFN-α	77	86	89
IFN-γ	53	66	52

28022

Human Parainfluenza Virus Type 3 Inhibits Interferon gamma-Induced MHC class II Expression Directly and by Inducing Interferon Type I

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Human parainfluenza virus type 3 (HPIV3) establishes persistent infection in cultured cells and in humans under some conditions. Several viruses have been shown to down-regulate IFN-γ-mediated MHC class II expression, which is believed to be one of the diverse mechanisms by which they elude immunosurveillance to establish persistence. In this communication, we show that HPIV3 strongly inhibits the IFN-γ-induced MHC class II expression but not MHC class I, in HT1080 human fibrosarcoma cells. The culture supernatant of HPIV3-infected cells also inhibited IFN-γ-induced MHC class II, a phenomenon that was found to be due, in large part, to IFN type I. The expression of another IFN-γ-inducible gene, ICAM-1 was not affected in HPIV3-infected cells, showing that the HPIV3 inhibitory effect was specific to MHC class II. STAT1 activation was not affected by HPIV3 at early postinfection times and was only partially inhibited at later times. These data suggested that the potent inhibition of MHC class II expression was due to a defect downstream of STAT1 activation in the IFN-γ-induced MHC class II expression pathway. CIITA is the unique mediator of IFN-γ-induced transcription from the MHC class II promoter. By RNase protection analysis, CIITA expression was found to be strongly inhibited in HPIV3-infected cells. The culture supernatant containing IFN type I, on the other hand, inhibited MHC class II expression without affecting CIITA expression. These data indicate that HPIV3 inhibits IFN-γ-induced MHC class II expression primarily by the viral gene products targeting CIITA and additionally by inducing IFN type I to target one or more steps further downstream.

28024

REGULATION OF IL-18 PRODUCTION FOLLOWING UVB IRRADIATION OF HUMAN KERATINOCYTES

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IL-18, first designated as IFN-γ inducing factor, is a newly identified cytokine synthesized by numerous cell-types. Accumulating evidence suggests that IL-18 is a pro-inflammatory cytokine widely involved in both systemic and local inflammatory mechanisms. However, the regulation of this cytokine is not yet well understood. In the present study, we have investigated the mechanisms of IL-18 regulation in human keratinocyte cultures (cell line NCTC 2544) exposed to ultraviolet B (UVB) radiation. Using a semi-quantitative RT-PCR protocol, we have observed a significant increase of IL-18 mRNA transcript levels in UVB-irradiated NCTC 2544 cell. In the same way, we have found an augmentation of IL-18 protein release in the UVB-exposed NCTC 2544 cell culture supernatants. We also investigated involvement of NF-κB in the mechanisms of IL-18 production. For this study, NCTC 2544 cell cultures were treated with curcumin, an inhibitor of NF-κB activation. Treatment of NCTC 2544 cell cultures with curcumin results in an increase of both mRNA transcripts and protein secretion. On the other hand, UVB irradiation of NCTC 2544 cell cultures before treatment with curcumin induces a decrease of IL-18 synthesis. Using a nuclear run on assay we have confirmed, in our experimental conditions, the implication of NF-κB in IL-18 regulation. These results support 1/ the role of IL-18 in inflammatory effects of UVB radiation, 2/ the implication of NF-κB in IL-18 regulation, and 3/ the differential effect of curcumin to regulate IL-18 synthesis depending on the activation state of keratinocyte cell cultures.

28026

INDUCTION OF APOPTOSIS IN HERPESVIRUS LYMPHOMAS BY THE ANTIVIRALS INTERFERON ALPHA AND AZIDOTHYMIDINE

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Azidothymidine (AZT) alone induced apoptosis in AIDS related Epstein Barr Virus positive Burkitt's lymphomas (EBV+ BL) and parenteral AZT and Interferon alpha (IFN α) induced tumor regression in EBV+ BL but not EBV- BL. IFN α alone or in combination with AZT had no effect on primary EBV+ BL lines. In contrast to EBV+ BL, the combination of AZT and IFN α induced significant apoptosis in Primary Effusion Lymphoma (PEL) cells whereas neither agent alone had activity. We found that in the PEL cells BCBL-1 and BC-3, these agents induced cleavage of FLICE. This suggested that AZT and IFN α mediated cytotoxicity in PEL was associated with death receptor (DR)/DR ligand mediated apoptosis. Further investigation demonstrated that in PEL, IFN α induced the DR ligand, TRAIL, an effect not observed in EBV+ BL lines. To confirm the role of TRAIL in apoptosis in PEL, we transfected BCBL-1 cells with dominant negative FADD (dnFADD). Clones expressing the mutant protein were analyzed for sensitivity to AZT and IFN α and the conventional chemotherapeutic agent, etoposide. AZT and IFN α mediated apoptosis was inhibited in BCBL-1 (dnFADD) cells, however sensitivity to etoposide was retained. AZT also synergized with soluble TRAIL to induce apoptosis in PEL cells. Non-thymidine analogue antiviral nucleosides were completely inactive in both EBV+ BL and in combination with IFN α in PEL. Our data indicates that thymidine nucleosides promote signaling through death receptors and synergize with IFN α (TRAIL) to induce a suicide program in HHV-8 + PEL. This represents a potentially powerful anti-tumor combination.

28028

ROLE OF UREASE AND HSP60 IN MUCOSAL INFLAMMATION ACCOMPANIED WITH H. PYLORI INFECTION

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Cytokines have been proposed to play an important role in *Helicobacter pylori (H. pylori)* - associated gastroduodenal diseases, but the exact mechanism of the cytokine induction still remains unclear. *H. pylori* urease and hsp60 are considered to be one of the virulence factors for the inflammation in the gastric mucosa that occurs on *H. pylori* infection. However, the response of human gastric epithelial cells to the stimulation of urease and hsp60 has not been investigated. In the present study, we used human gastric epithelial cells in a primary culture system and examined whether *H. pylori* urease and hsp60 stimulate the gastric epithelial cells to induce proinflammatory cytokines by using RT-PCR and ELISA. First, in peripheral blood mononuclear cells (PBMC) and gastric cancer cell line (MKN-45 cells), we confirmed the ability of purified *H. pylori* urease and hsp60 to induce the production of proinflammatory cytokines. Furthermore, we demonstrated that the human gastric epithelial cells produced interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), but not IL-8 following stimulation with urease. On the other hand, the patterns of cytokine production differed among human PBMC, MKN-45 cells, and human gastric epithelial cells. These results suggest that the human gastric epithelial cells contribute to the induction of proinflammatory cytokines by the stimulation of *H. pylori* urease and hsp60.

28018

RECOMBINANT INTERFERON ALPHA-2B APPLICATION COMBINED WITH ANTIOXIDANTS IN PERINATAL VIRAL HEPATITIDES TREATMENT

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43 newborns with suspicion on hepatitis aged from 9 days to 3 months have been observed. To verify the diagnisis biochemical data of liver functions and viral serological markers were tested with ensyme immunoassay method, specific viral acids were tested with PCR method. 17 cases of the illness were etiologically related with hepatitis viruses (8 cases - HBV, 9 cases - HCV), 26 cases were associated with specific intrauterine infections (cytomegalia, toxoplasmosis, listeriosis, syphilis). The specific markers of viral hepatitides (HBsAg, DNA HBV, IgM+IgG Hbcor, RNA HCV, anti-HCV, anti-CMV IgG, anti-CMV IgM) were observed within 3 first months of life, so perinatal mechanism of infection may be supposed. Patients did not take blood medicine. In most cases HBV, HCV were primary chronic with high level transaminases activity and UZ liver changes, one patient suffered fulminant hepatitis.

7 pregnant mothers suffered acute HBV hepatitis, one mother was diagnosed ill with chronic B hepatitis. All mothers of perinatally infected children HCV were ill with chronic hepatitis.

Dynamics of the IFN-status and indexes of immunity of 29 patients (6-HB, 7- HC, 16-CMV-infected) were analysed, and on the basis of these researches the patients were treated with individual schemes of recombinant alpha-2b interferon combined with antioxidants in suppositoria (viferon), 500 000 ME dayly for 3-10 months with summary dose of interferon 28 -130 millions ME. CMV-hepatitis were treated with lower doses for shorter courses.9 patients (69%) with viral hepatitis reached stable remission (4 of 6 HB patients, 5 of 7 HC patients). Neutralizing antiviral IFN activity antibodies have not been produsedeven after prolonged observation (2 years).Interferon alpha-2b with antioxidants in suppositoria is convenient in application, causing no side effects. No remission was observed without interferon (4 cases). Within the first year of age all perinatally CMV-hepatitis children recovered.

28009

INHIBITORY AND PROMOTING ACTIVITY OF VIRUS-INDUCED INTERFERON TOWARD BORNA DISEASE VIRUS IN MICE

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Borna disease virus (BDV) is a non-cytolytic RNA virus that can replicate in neuronal cells of mice. We previously showed that transgenically expressed IFN-α1 can effectively block BDV multiplication in the CNS. To determine whether IFN which may be induced during the course of infection can similarly help to restrict viral spread, we compared the BDV growth kinetics in cultured cells and in brains of wild-type and IFNAR$^{o/o}$ mice that lack a functional type I IFN receptor. As expected if virus-induced IFN exhibited antiviral activity, BDV replicated well in embryo cells from IFNAR$^{o/o}$ but not wild-type mice. However, immunohistochemistry and western blot analysis showed that virus spread was not accelerated in brains of infected IFNAR$^{o/o}$ mice and that viral antigen persisted at high levels in both wild-type and mutant mice, suggesting that the IFN response after BDV infection was too weak or occurred too late for being effective. Surprisingly, Northern blot analyses with strand-specific hybridization probes showed that viral transcript levels in brains of persistently infected IFNAR$^{o/o}$ mice were about 10-fold lower than in congenic wild-type 129 mice, whereas viral genomic RNA was produced in large excess in brains of IFNAR$^{o/o}$ mice. Thus, IFN action in persistently infected neuronal cells seems to freeze the BDV polymerase in transcription mode which results in enhanced viral mRNA synthesis and low genome replication. To our knowledge, this is the first report demonstrating that IFN can stimulate (rather than inhibit) viral mRNA synthesis.

28027

Listeria monocytogenes activates endothelial cells by a listeriolysin O-dependent pathway: Activation of NF-κB and upregulation of adhesion molecules and chemokines.

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Listeria monocytogenes, is an invasive facultative intracellular bacterium which crosses the vascular endothelium and disseminates to the placenta and the central nervous system. Its interaction with endothelial cells is crucial for the pathogenesis of listeriosis. By infecting *in vitro* human umbilical vein endothelial cells (HUVEC) with *L. monocytogenes*, we found that wild-type bacteria induced the expression of the adhesion molecules (ICAM-1 and E-selectin), chemokine secretion (IL-8 and MCP-1) and NF-κB activation. The activation of HUVEC required viable bacteria and was abolished in *prfA*-deficient mutants of *L. monocytogenes*, suggesting that virulence genes are associated with endothelial cell activation. By a genetic approach using mutants of virulence genes, we found that listeriolysin O (LLO)-deficient mutants inactivated in the *hly* gene did not induce HUVEC activation, as opposed to mutants inactivated in the other virulence genes. Adhesion molecule expression, chemokine secretion and NF-κB activation were fully restored by a strain of *Listeria innocua* transformed with the *hly* gene encoding LLO. The relevance *in vivo* of endothelial cell activation for listerial pathogenesis was investigated in transgenic mice carrying an NF-κB responsive *lacZ* reporter gene. NF-κB activation was visualized by a strong *lacZ* expression in endothelial cells of capillaries of mice infected with a virulent hemolytic strain, but was not seen in those infected with a nonhemolytic isogenic mutant. Direct evidence that LLO is involved in NF-κB activation in transgenic mice was provided by injecting intravenously purified LLO, thus inducing stimulation of NF- κB in endothelial cells of blood capillaries. Our results demonstrate that functional LLOsecreted by bacteria contributes as a potent inflammatory stimulus to inducing endothelial cell activation during the infectious process.

28017

Plasmid DNA Encoding Murine IFN-α1 Administered Intravaginally Post Infection Reduces Mortality of Herpes Simplex Virus Type 2 (HSV-2)-Infected Mice.

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A study was undertaken to determine the efficacy of a plasmid DNA encoding murine IFN-α1 introduced into the vaginal lumen of mice post infection. The administration of plasmid DNA encoding IFN-α1 into the vaginal lumen enhanced MHC class I gene expression 41%. Mice infected with HSV-2 and treated intravaginally 24 hr later with 100 μg DNA encoding IFN-α1 showed enhanced survival (10/15) in comparison to mice treated with plasmid DNA vector alone (3/10) or vehicle (4/27). The protective effect was time-dependent and corresponded with a reduction in viral antigen expressed in the vaginal epithelium, IFN-γ tissue levels, and replicating virus recovered from vaginal tissue early (day 3 post infection) during the infection. By day 7 post infection, HSV-2 glycoprotein B transcript expression was no longer detectable in vaginal tissue from the IFN-α1 transgene-treated group (0/8) compared to levels expressed in plasmid vector-treated controls (4/6). Although there was a significant increase in both CD4 and RANTES transcript expression in the vaginal tissue 3 days post infection, there were no differences in the levels comparing the DNA-treated groups. CD8 transcript levels were reduced in the IFN-α1 transgene-treated group in comparison to the vector controls day 7 post infection. Whereas additional transcript levels were detected (IL-6, iNOS, and TGF-β), others were noticeably low (IL-4) or absent (IL-10) in vaginal tissue from HSV-2 infected mice. Collectively, the results suggest the application of DNA encoding type I IFN is an effective and alternative approach to control vaginal HSV-2 infection by antagonizing viral replication.

28016

ACTIVATION OF SIGNAL TRANSDUCTION AND APOPTOSIS IN HEALTHY LYMPHOMONOCYTES EXPOSED TO BYSTANDER HIV-1 INFECTED CELLS

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Background. A major hallmark of HIV-1 infection is persistent activation of the immune system, that leads to altered cytokine production and increased apoptosis.
Aim: To analyze the induction of factors involved in cytokine signal transduction, such as STAT 1 proteins and IRF-1 mRNA, and the induction of apoptosis, in normal PBMC exposed to HIV-infected cells
Results: Western blot analyses and RT-PCR results indicate that both cells infected with a X4 strain and cells infected with a R5 strain are able to increase intracellular levels of STAT 1 α and β proteins as well as IRF-1 mRNA. This effect was prevented by neutralizing antibodies against IFN-α. HIV-1 infected cells dose-dependently induced apoptotic commitment in normal PBMC, as revealed by DNA fragmentation analysis, but this was not accompanied by an increase of caspase-3 proteolytic activity, even if HIV-infected cells were able to slightly up-regulate ICE mRNA. Apoptosis induction could be abrogated mainly by antibodies directed to TNF-α and, at a lower extent, by antibodies to IFN-γ.
Discussion: All these findings suggest that uninfected PBMC can undergo activation of signal transduction and apoptosis after exposure to bystander HIV-infected cells subsequent to the induction of cytokines such as IFNs and TNF-α.

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28015

INFLUENCE OF COSTIMULATORY MOLECULES ON THE IMMUNE RESPONSE TO *LEISHMANIA MAJOR* BY HUMAN CELLS *IN VITRO*.

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Costimulatory molecules play an essential role in the activation and maintenance of T cell responses. Using an in vitro system in which human PBL were stimulated with autologous *Leishmania major*-infected macrophages, we studied the influence of different costimulatory molecules on APC and their interactions with T cells. We observed that B7-mediated costimulation is required for the development of an initial immune response, and that B7-2 is the principal B7 molecule for initiation of T cell activation. Blockade of B7 inhibited the production of IFN-γ, IL-5, and IL-12. In addition, we found that high levels of B7-2, but not B7-1, are constitutively expressed by macrophages. The blockade of CD40-CD40L interaction also inhibited the production of cytokines, such as IFN-γ, IL-5 and IL-12, but not IL-10. Infection of macrophages with *L. major* by itself did not induce changes in the expression of B7-1, B7-2 or CD40. However, the expression of B7-1 and CD40 was up regulated on macrophages after culture in the presence of PBL. Because IFN-γ is among the most important anti-*Leishmania* T cell cytokines, and IFN-γ is responsible for the activation of parasitotoxic macrophages, we explored the cellular origin of IFN-γ and found that it is produced by a higher percentage of $CD8^+$ (5.4%) than $CD4^+$ cells (3.1%). Finally, we found that IL-12 increased the percentage of both subpopulations producing IFN-γ (up 14% for $CD8^+$ and 5% for $CD4^+$ cells). We have also observed the role of costimulatory molecules using PBMC from tegumentar leishmaniasis patients, stimulated in vitro with soluble antigen of *Leishmania*. Preliminary results showed a partial inhibition in the production of IL-2, IL-10 and TNF-α, but no differences were observed in IFN-γ secretion, suggesting a differential immunomodulation induced by *Leishmania*.

28014

STRONG TH1 CYTOKINE PATTERN INDUCED BY AN MENINGOCOCCAL VACCINE IN DIFFERENT MICE STRAINS

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In previous studies we demonstrated that the Cuban outer membrane vesicle based antimeningoccocal vaccine (VA-MENGOC-BC) induced a Th1 cytokine pattern when is administrated to adult humans, response that may explained the high efficacy and protection induced by this vaccine. Nevertheless, the characterization of the Th1/Th2 pattern induced by VA-MENGOC-BC in Balb/c and C57BL6 and the effect of a previous Th2 cytokine environment induced by Leishmania mayor on the response induced by outer membrane vesicles (OMV), the main constituent of VA-MENGOC-BCÒ is not known. In the present work the cytokine pattern and the IgG subclass induced after two dose of VA-MENGOC-BC was determined. Spleen cells were isolated and re-stimulated in vitro with OMV. The culture media were assayed for IFNg and IL-5 using ELISA techniques. IgG1 and IgG2a subclass against OMV in sera were also evaluated. The production of high levels of IFNg and IgG2a was demonstrated in both mice strains. In addition, Balb/c mice were pre-infected with L. major and were immunized with OMV 10 days later. Result showed that Th2 cytokine profile were established in mice infected with L. major, but these environment did not affect the production of IgG2a induced when OMV were administrated. In conclusion, these evidences suggest that this vaccine induce a strong Th1 response, also in mice.

28013

IMMUNISATION OF MICE WITH DNA ENCODING TYPE I IFN SUBTYPES PROTECTS AGAINST CYTOMEGALOVIRUS INFECTION AND DISEASE

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Cytomegalovirus (CMV) is a member of the family of herpesviruses. Human CMV (HCMV) is known to infect 60-80% of individuals in developed countries while virtually 100% of the population of under-developed countries is affected. Although, the majority of these infections are asymptomatic. CMV is regarded as an important pathogen in immunocompromised hosts (transplant recipients, AIDS patients) or those with an immature immune system (foetus, neonates). Intra-uterine CMV infections are second only to Downs Syndrome as a known cause of mental retardation. CMV infection is also known to cause myocarditis, an inflammatory disease characterised by cellular infiltration and necrosis of the heart. Due to the strict host-specificity of CMV we are unable to study viral pathogenesis and disease in a direct model, however, murine cytomegalovirus (MCMV) infection of mice has been established as a model for HCMV. We have investigated the use of prophylactic administration of naked DNA plasmids encoding type I IFNs subtypes (IFNα1, α2, α4, α5, α6, α9, β) in the MCMV model. MCMV replication was monitored in target tissues and the development of acute myocarditis was determined in DNA-immunised mice challenged with MCMV. The IFN subtypes were then analysed in a comparative study to determine their efficacies in MCMV infection and myocarditis. The IFN subtypes exhibited a differential effect on both viral infection and disease. These studies highlight the potential for improved efficacy of type I IFNs in therapeutic applications by the appropriate use of IFN subtypes.

28012

INNATE IMMUNITY TO TRANSMISSION OF HIV: PREVENTION OF SEXUAL TRANSMISSION

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There are 34 million carriers of HIV and 6 million transmissions each year. Most of the transmissions are by sexual contact. Mucosal transmission of HIV occurs mainly via infected leukocytes. Oral transmission is prevented by the innate immunity of saliva, which inactivates HIV-infected leukocytes (Arch Intern Med. 1999;159:303-310; JID 2000; 181:498-504). Vaginal and rectal transmission is frequent and probably is mainly due to the survival of HIV-infected leukocytes in seminal fluid. We studied the possible adaptation of oral resistance mechanisms to protect the vulnerable vaginal and rectal mucosal sites of transmission. To prevent HIV transmission vaginally and rectally, substances that mimic the salivary defense by targeting the transmitting HIV-infected leukocytes are needed. Previously used preventives containing the surfactant nonoxynol-9 are ineffective in humans, probably because of mucosal inflammation. Therefore, less irritating anticellular substances are needed to prevent transmission by the HIV-infected leukocytes in seminal fluid. Experimentally, HIV-infected human leukocytes or cell-free HIV in seminal fluid were treated with candidate preparations including FDA-approved, over-the counter vaginal lubricants (Astroglide, Vagisil and ViAmor), bile salts, and disinfectants for blood products. Some of these substances inhibit lymphocyte production of HIV by >1,000-fold in 15-30 minutes, as well as inhibiting cell-free HIV. Medical application of such safe FDA-approved products or their active components may interdict sexual transmission of HIV. Also, these products may be cost effective in underdeveloped countries. Clinical trials may be indicated.

28010

LIVER SPECIFIC IFNα2 GENE EXPRESSION RESULTS IN PROTECTION FROM INDUCED HEPATITIS

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The current therapy for hepatitis B and C is based on systemic administration of recombinant human interferon-α (rIFNα). However, this systemic delivery of r-hIFNα is associated with severe side effects, but more importantly, it is effective only in a small percentage of patients. In an effort to maximize IFNα antiviral efficacy, we have explored the therapeutic potential of murine IFNα2 (mIFNα2) selectively expressed in the liver. To this end, we have developed a helper-dependent adenovirus vector (HD) containing the mIFNα2 gene under the control of the liver specific transthyretin promoter (HD-IFN). Comparison with a first generation adenovirus carrying the same mIFNα2 expression cassette, indicates that at certain HD-IFN doses the induction of antiviral genes can be achieved in the absence of detectable circulating mIFNα2. Challenge of injected mice with mouse hepatitis virus MHV-3 showed that HD-IFN provides high liver protection. Moreover, liver protection was also observed in non-viral acute hepatitis induced by Concanavalin A (ConA) at 1 month p.i. These results hold promise for the development of a gene therapy treatment of chronic viral hepatitis based on liver-restricted expression of IFNα2

28025

Herpes Simplex Virus Type I ICP0 Null Mutant is Sensitive to Type I But Not Type II IFN: Resitance to IFN Can Be Restored By Supplementing ICP0.

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Introduction: Type I interferons (IFN-α and -β) are a key innate immune component that promotes anti-viral resistance in cells at the transcriptional and post-transcriptional level. Herpes simplex virus I (HSV-1) ICP0 is an immediate early protein with pluripotent properties such as transcriptional activation, inhibition of cell cycle progression and reactivation from latency. The use of HSV-1 mutants allows a direct analysis of viral replication under a controlled environment. **Methods:** To this end, replication of the ICP0 null mutant 7134, which lacks both genes encoding for the immediate early protein 0 (ICP0), was studied in the presence and absence of type I and type II IFNs using plaque reduction assays. HSV-1 KOS is the parental virus strain of 7134 and Vero cells are the parental cell line of L7 which express ICP0 under an HSV-1 promotor. **Results:** Type I IFNs strongly reduce replication of the ICP0 mutant whereas type II IFN (IFN-γ) does not. This functional IFN-resistance property of ICP0 cannot be overcome by infections with high MOI (MOI=0.1-10). Replication of 7134 in Vero-cells was inhibited by IFN-α (IC_{50}=15.0 U/ml) and -β (IC_{50}=4.3 U/ml) in a dose-dependent fashion. At high concentrations (1000 U/ml) of type I IFN 7134 replication was reduced about 1000-fold compared to a 10-fold reduction of the parental strain KOS. IFN resistance could be restored to levels similar to wild-type (KOS) by supplementing ICP0 through the complementing cell line (L7) or through coinfection with adenoviral constructs expressing ICP0 under a doxycyclin promotor. ICP0 does not confer resistance to the IFN-sensitive Vesicular stomatitis virus (VSV). **Conclusion:** The results suggest that the expression of the ICP0 genes confers resistance to type I but not type II IFNs in HSV-1 in an MOI independent fashion. This is a novel mechanism of HSV-1 to circumvent an innate immune mechanism. ICP0 does not induce an IFN resistant phenotype in VSV. Dr. Peter Härle is a recipient of a research fellowship from the Deutsche Forschungs Gesellschaft (HA2993/1-1).

28011

BCG RE-VACCINATION IN SCHOOL AGE CHILDREN: CORRELATION BETWEEN SURROGATE PROTECTION INDICATORS.

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Vaccination with BCG has long been applied worldwide to prevent tuberculosis. However, its degree of protection against each of the forms of disease ranges from null to 90 percent in the literature. Clinical trials for a substitute vaccine will be long and costly. In this work, we have studied the evolution of immunological parameters which constitute potential surrogate markers of protection against the disease in a group of school age children re-vaccinated in Salvador (Bahia, Brazil). One hundred thirty-seven school children were vaccinated with BCG (Moreau-RJ strain). Children that did not present scar indicative of previous BCG vaccination were not included in this study. Tuberculin reactivity pre- and 60 days post-vaccination was determined independently by two experienced subjects. At the same time points approximately 50 l of blood was collected by digital puncture, diluted in RPMI and cultivated for 3 days with *Mycobacterium tuberculosis* crude extract. Culture supernatants were assessed for IFN, TNF and IL-10 levels. Individuals were grouped according to the extent and conversion of the tuberculin test reaction. No correlation was found between PPD reactivity or conversion and cytokine response pre or post vaccination. Interestingly, high producers of IFN (75% quartile; 1872.0996,6) post-vaccination were found to correspond to individuals with increased IFN production and low IL-10 levels upon BCG administration, while the inverse was observed for low IFN producers (25% quartile, 0.00.0). Our results suggest a heterogeneous response to BCG re-vaccination, with possible implications in vaccine protection, which is independent of tuberculin test reactivity. Support: CNPq, PRONEX.

28029

THE ROLE OF AN AVIAN IL-8-LIKE CYTOKINE IN THE RECRUITMENT OF *SALMONELLA*-IMMUNE LYMPHOKINE-ACTIVATED HETEROPHILS TO THE SITE OF BACTERIAL INVASION

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Increased resistance to *Salmonella enteritidis* (SE) organ infectivity in chickens can be conferred by the prophylactic administration of SE-immune lymphokines (SILK). Resistance is associated with an enhanced heterophilic accumulation within 4 hours of SILK injection. In these studies, we investigated the possible role of IL-8 in SILK-mediated heterophil recruitment during SE infections in young chickens. Heterophil accumulation was enhanced 2-4 h after the i.p. injection of both SILK and SE (SILK/SE) when compared to the control chicks. An i.p. injection of a rabbit polyclonal anti-human IL-8 antibody significantly ($P < .01$) reduced the accumulation of heterophils in the peritoneum after the injection of SILK/SE. Injections of preimmune rabbit IgG had no effect on peritoneal heterophil numbers. Within 2 h of injection of SILK/SE, a ten-fold increase in heterophil chemotactic activity was found in the peritoneal lavage fluid from these chicks compared to the saline control chicks. Pretreatment, with the anti-IL-8 antibody, of the peritoneal lavage fluids collected from the SILK/SE-treated chicks dramatically reduced this heterophil chemotactic activity. Treatment of the lavage fluids from all groups with preimmune IgG had no effect on heterophil chemotaxis. Additionally, pretreatment of SILK with the anti-human IL-8 antibody had no effect on heterophil chemotaxis. The results from these experiments suggest that IL-8 is produced locally by the host in response to both the SE infection and SILK. With these studies, we have established that IL-8 is a major chemotactic factor produced by the host that aids in mediating ILK/SE-induced recruitment of heterophils to the site of SE invasion.

28065

Measles and Vesicular Stomatitus Virus Activation of IRF-3

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The cellular response to viral infection stems from the activation of multiple signaling pathways leading to the transcriptional activation of anti-viral genes. Among the transcription factors implicated in the immediate early antiviral response is the interferon regulatory factor 3 (IRF-3) protein. Upon virus infection, IRF-3 is post translationally modified through the phosphorylation of serine residues in the C-terminus allowing it to dimerize, translocate to the nucleus, bind DNA, and associate with CBP. Binding of IRF-3 occurs on the positive regulatory domain III (PRDIII) of the Interferon-β (IFN-β) gene resulting in the recruitment of CBP and the transcriptional activation of anti-viral genes including type 1 IFNs and chemokines. IRF-3 has been found to be activated by several types of viruses such as reovirus, cytomegalovirus (CMV), sendai virus (SeV), and newcastle disease virus (NDV). In a search to find additional activators of IRF-3, both measles virus (MV) and vesicular stomatitus virus (VSV) were found to induce IRF-3 activation in human embryonic kidney cells (HEK293) and in human bronchial epithelial cells (A549). Like the previously documented viral activators, MV and VSV infection induced the phosphorylation of IRF-3 resulting in DNA binding, and transactivation of RANTES and IFN-β. As shown for SeV, overexpression of the dominant negative form of IRF-3 which lacks the DNA binding domain (IRF-3 ΔN), reduced the expression of RANTES to basal level, compared to a 5 fold induction during wild-type infection of MV and VSV. The kinetics of IRF-3 activation occurred between 18-24 h at a multiplicity of infection of 1.0, suggesting that the signal for IRF-3 activation may be the result of the viral switch from transcription to replication. Although double stranded RNA (dsRNA) has been implicated in the activation of IRF-3, we found dsRNA necessary, but not sufficient, to induce IRF-3 binding to the PRD III element. This data then implicates the need for an additional viral signal that may be common amongst all the viral activators that will be recognized by the cell, leading to the phosphorylation and activation of IRF-3.

28050

COMPARISON OF IFN-τ AND IFN-α EFFECTS ON THE EXPRESSION OF 2',5'-OAS, PKR AND MxA PROTEIN IN MACROPHAGES: CORRELATION WITH ANTI-HIV ACTIVITY

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Type I interferon (IFN) are cytokines which exhibit efficient antiviral activities notably against HIV. IFN-α is used as a treatment in HIV-infected patients with Kaposi's sarcoma, but its severe side effects have restricted its clinical uses. IFN-τ is a ovine or bovine non-cytotoxic type I IFN which displays higher inhibitory effects towards HIV replication than IFN-α, particularly in monocyte-derived macrophages (MDM). These effects were correlated with the inhibition of HIV proviral DNA synthesis and with combinatory effects with IL-6: IL-6 mRNA expression and IL-6 synthesis are increased in dose-dependent manner in MDM treated with IFN-τ and the antiretroviral activity of this IFN was reduced when IL-6 biological activity was inhibited by specific anti-IL-6 mAb. In this study, several events were first explored in early steps of HIV biological cycle: IFN-τ inhibits the synthesis of early and intermediate RT products in MDM but less efficiently than IFN-α. In contrast, we evidence that the induction of 2',5'-OAS, PKR and the MxA protein mRNA in MDM appears to be stronger than the one seen after IFN-α treatment. Moreover, a co-treatment with IFN-τ and IL-6 seems to reduce the inductions obtained with IFN-τ alone and does not increase the inhibition of the synthesis of RT products as seen for IFN-α, whereas specific anti-IL-6 does not affect it.

Altogether, these results show that IFN-τ certainly uses the same pathways as other type I IFN, and that differences observed between IFN-τ and IFN-α could explain those in anti-HIV activity. On the other hand, the precise role of IL-6 and its level of action with IFN-τ still remains unclear.

28051

HSV-1 REPRODUCTION IN CULTURED HUMAN ENDOTHELIAL CELLS. THE EFFECT OF ENVIRONMENT CELLS PRODUCED SOLUBLE FACTORS.

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The ability of intimal smooth muscle cells (SMCs) and blood leukocytes to control the development of HSV-1 infection in human vascular endothelial cells (ECs) was studied.

The cultured ECs support HSV-1 reproduction. Human endothelium is highly heterogeneous morphologically and functionally. EC subcultures isolated by cloning techniques from primary human aortic EC cultures were investigated. EC subcultures have shown different sensitivity to HSV-1 reproduction, also as diverse profile of cytokines production. Co-culture of ECs with SMCs growing in TransWell inserts significantly delayed the development of signs of virus reproduction. Results varied and possibly depended on heterogeneous peculiarity of ECs. At a MOI 0.001, SMC-conditioned media treatment leaded to the establishment of latent infection in ECs: this infection could persist in a subculture of infected cells and re-activated by substitution of conditioned medium by standard EC growing medium. The decrease of virus infection in ECs was also obtained when the cells were cultured in human peripheral blood leukocytes conditioned medium (PBLCM), containing numerous cytokines. Visual signs of infection and virus reproduction have not been found after infection at a MOI 0.001 and PBLCM treatment of ECs. Separately or jointly used cytokines (IL-1, TNFα, G-CSF) only delayed the virus reproduction.

The results suggest that soluble factors secreted by resident cellular components of the vascular intima (SMCs) and leukocytes may be involved in mechanism of endothelium-virus interaction and modify the pathogenesis of HSV-1 disease.

28052

THE ROLE OF IL-16 IN INFLAMMATORY BOWEL DISEASE

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Interleukin (IL)-16 is produced by a variety of immune (T cells, eosinophils, and dendritic cells) and non-immune cells (epithelial cells, fibroblasts). It has been characterized as a chemoattractant for $CD4^+$ cells. Recently it was shown that IL-16 contributes to the regulation of cell recruitment and activation during inflammatory diseases such as asthma or inflammatory bowel disease (IBD). In concert to this IL-16 activates the expression of proinflammatory cytokines (IL-1β, IL-6, TNF-α, IL-15). CD4 was demonstrated to be a specific receptor for IL-16 although ligation of IL-16 to CD4 seems not to be neccessary in every case to exert its biological activities. We investigated the expression of IL-16 in colonic biopsies from patients with IBD. IL-16 mRNA and protein amounts were significantly increased in IBD samples as compared to normal control individuals and disease specificity controls. Immunostainings of the same specimens supported our results. Moreover, eosinophils appeared to be the main producers of IL-16 in the inflamed colonic mucosa. Increased IL-16 expression was only detected in inflamed colonic regions. The role of IL-16 in IBD is not yet clear. Recruitment of $CD4^+$ cells could be one possibility. In order to test the ability of IL-16 to activate the secretion of pro-inflammatory cytokines and chemokines peripheral blood monocytes were isolated and incubated with IL-16. The secretion of cytokines such as TNFα, IL-1β, and IL-6 and the chemokine MCP-1 was significantly increased approximately 3-5 fold. Among others the expression of these proteins is known to be upregulated in IBD. We conclude that IL-16 could contribute to the pathogenesis of IBD by regulating the expression of pro-inflammatory cytokines in monocytes.

28053

COMPARATIVE SENSITIVITY OF INDUCTION OF THE INTERFERON (IFN) SYSTEM IN MICE

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Double-stranded RNA (dsRNA) treatment, which mimics certain aspects of viral infection, is known to induce the production of Type I IFN. Interestingly, dsRNA can also exert direct effects on the transcription rate of interferon-inducible genes in addition to the indirect impact on many of the same genes through the secondary effects of autocrine or paracrine IFN. Little is known of the relative sensitivity of these inductions *in vivo*. We studied, in mice, the limiting concentrations of poly I:CLC (a synthetic dsRNA preparation) required for induction of (a) IFN in serum, (b) the IFN response gene mRNA ISG54 in spleen and liver, (c) the IFNβ mRNA in spleen, and (d) resistance of mice to Banzi virus infection. The results showed that, for detection of induction of the components of the IFN system, resistance to infection was seven-fold more sensitive than ISG54 mRNA, and seventy-fold more sensitive than either IFNβ mRNA or IFN production in serum. We may conclude that for *in vivo* screening of IFN-inducers like poly I:CLC, the order of highest to lowest sensitivity is resistance to infection, ISG54 mRNA, and then either IFNβ mRNA or serum IFN. Thus protection against viral challenge is a more sensitive readout than measuring transcriptional endpoints or IFN production in culture supernatants. We may also hypothesize that, *in vivo*, the IFN effector molecules for antiviral activity are more easily detectable than IFN itself, perhaps because they are more durable after induction.

28055

MICROARRAY ANALYSES REVEALED PARTIALLY OVERLAPPING SETS OF HUMAN GENES INDUCED BY DOUBLE-STRANDED RNA. IFN-β, TNF-α AND IL-1

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Viral infection of mammalian cells causes immediate and profound changes in the patterns of cellular gene expression. Often such changes are brought about by transcriptional up- or down-regulation of specific cellular genes by viral double-stranded RNA. In the current study, we have used cDNA microarray analyses for identifying human dsRNA-stimulated genes (DSG). Screening of 4600 cDNA and EST clones produced 113 candidate DSGs. Detailed characterization of 8 of them by Northern blotting revealed that all of them were strongly induced by dsRNA. Induction of some mRNAs peaked at 1 h whereas that of others peaked at 6 h and declined thereafter. For all but one, the induction process did not need ongoing protein synthesis. Transcription of some DSGs was also induced by different cytokines. One gene was induced by dsRNA, IFN-β, TNF-α or IL-1; another by dsRNA, TNF-α or IL-1, but not IFN-β; a third one by dsRNA or IFN-β only and a fourth gene by only dsRNA. Functionally, the family of DSGs included genes involved in cell signaling, apoptosis, cell cycle regulation, transport, RNA synthesis, protein synthesis and processing, cell metabolism and cell structure. Thus, dsRNA can regulate a broad range of cellular activities in a quick, potent, specific and transient manner.

28057

Vitiligo Associated with Alpha - Interferon in two Patients with Chronic Active Hepatitis B

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We present two alpha-interferon treated patients with chronic hepatitis B who experienced induction and aggrevation of vitiligo patches respectively as the complication of the treatment. The first case was a 21-year-old man who experienced emergence of small vitiligo patches on his hands during alpha-interferon therapy due to chronic ative hepatitis B. His patches diminished by itself after 6 months of the treatment with inferferon. The second case was a 41-year-old man whose pre existing confined vitiligo progressed dramatically during alpha-interferon therapy of hepatitis B. His aggrevrated vitiligo patches remained after treatment. Although interferons have been widely used in hepatitis for many years, only few reports have been recently published on emergence of vitiligo as a rare side effect of this drug in hepatitis C patients. To our knowledge we present the first report on vitiligo in hepatitis B patients treated with Alpha-interferon. Regrading immunomodulating action of interferons as well as probable autoimmune pathogenesis of vitiligo, this skin complication of interferon therapy can be explained.

NOTE: The pathologic reports and photographs of the patients will be presented

28058

LOCAL IMMUNOTHERAPY OF INFLAMMATORY PROCESSES WITH HUMAN RECOMBINANT INTERLEUKIN-1 BETA

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Interleukin-1β (IL-1β) is a cytokine that plays key role in immune and inflammatory responses and is a good candidate for immunotherapy. Unfortunately systemic IL-1β administration causes various adverse effects due to activation of inflammatory response. The aim of this study was to investigate the possibility of local IL-1β application to avoid systemic toxicity and to study mechanisms of local IL-1β-induced immunostimulation. Recombinant human IL-1β has been used to treat patients with chronic bacterial lung abscesses, otitis and rhinosinusitis after failure of routine antibiotic therapy. IL-1β solution was applied directly to the inflammatory site daily for 5-10 days. This IL-1β local treatment led to a clinical improvement in majority of patients. Absolutely no systemic side effects were observed during IL-1β local therapy. IL-1β application caused an increase in initially depressed migration to fMLP, superoxide production, adgesion to plastic and phagocytosis of leukocytes isolated from the inflammatory sites. This effect was accompanied with significant IL-1-induced increase of IL-8 levels. Obtained results show that local IL-1 treatment can be used without toxic complications and with high effectivity for human immunotherapy.

28059

INHIBITION OF CYTOKINES, CHEMOKINES AND INTERFERONS BY ECTROMELIA VIRUS.

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The importance of cytokines in the host response to viral infection has been emphasized by the discovery of numerous viral gene products that inhibit their functions. The poxviruses are a family of large DNA viruses for whom cytokine blockade is a major immune evasion strategy. Outside the infected cell they express soluble, secreted receptors or binding proteins that sequester IL-1β (vIL-1βR), TNF (vTNFRs), chemokines (vCKBP), IFN-α/β (vIFN-α/βR), IFN-γ (vIFN-γR) and IL-18 (vIL-18BP). Inside the cell a viral homolog of eIF-2α and a viral dsRNA binding protein inhibit IFN-induced antiviral effects. We are studying ectromelia virus (EV), a poxvirus which causes mousepox, a severe disease of laboratory mice that previously served as a model for smallpox in humans. Usefully, the mousepox model permits study of the function of poxvirus immunomodulatory molecules during a natural infection. Previously, however, the expression of such molecules by EV was poorly characterised. Therefore we examined the expression and binding properties of the vIL-1βR, vCKBP, vIFN-α/βR, vIFN-γR and vTNFRs in 12 distinct EV isolates. EV expressed functional forms of all of these molecules, while the EV vIL-1βR and vIFN-γR displayed binding properties unique to this virus. The virus was also able to inhibit the intracellular, antiviral effects of IFN treatment efficiently. The expression of these immunomodulatory factors was conserved among the virus isolates which we studied, suggesting that they have a positive influence on the persistence of EV in its natural host. Study of their function using the mousepox model should provide insights into their roles *in vivo*.

28060

Influence of recombinant interferon$_{\alpha 2b}$ in gel (Viferon-gel®) on interferon status in children with often and prolonged diseases.

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Efficiency of the Viferon-gel® treatment was studied in 24 children 1 to 3 years old – 5 with stenosing laringotracheitis (SLT), 5 with recidivating obstructive bronchitis (ROB) and 14 with often and prolonged diseases (OPD). Interferon status was determined according to S.S. Grigoryan (1988). Level of activity of natural killers – activated NK (CD 16+8+) was determined by method of membrane immunofluorescence. Viferon-gel® (gene-engeneered α-2-β interferon in the form of gel, 40 000 IU/ml with vitamin E) was applied to orolaryngeal mucosa three times daily for one month with hard tampon.

Interferon status and activated NK (CD16+8+) dynamics

Index	*Nor-mal*	*SLT*		*ROB*		*OPD*	
		Before	*After*	*Before*	*after*	*before*	*Afte*
α-IFN	17.6± 1.3%	16.8± 1.2	19.5± 1.5	13.8±2	19.6± 1.4*	8.6± 1.2	18.5 0.9*
γ -IFN	15.4± 1.2%	4.54± 0.9	8.8± 1.1*	6.1± 0.2	7.9± 0.1	6.1± 1.6	9.4± 0.8*
Serum IFN	6.2± 0.1%	1.8± 0.2	2.8± 0.1	2.8± 0.1	3.0± 0.6	4.3± 0.5	4.8± 0.3
Activ. NK	1.4± 0.2%	1.2 ±0.3	0.8± 0.5*	0.8± 0.03	2.5± 0.1*	1.1± 0.1	3.3± 0.06

Note: * $p < 0,001$

Thus increased sensitivty to infectious diseases in children with SLT, ROB and OPD is associated with imperfect functioning of the interferon system, which requires therapy by interferon combined with antioxidant correction, in particular by Viferon-gel®.

28061

SPECTRUM OF VIRUS INHIBITION BY CONSENSUS INTERFERON YM643

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The spectrum of viruses inhibited by a genetically engineered consensus interferon YM643 (interferon alfacon-1) was evaluated using cytopathic effect inhibition assay or plaque inhibition assay for five DNA and twelve RNA viruses; this activity was compared with that of a natural interferon-α derived from lymphoblastoid cells (IFN-α [Namalwa]). The viruses inhibited by both IFNs were types 1 and 2 herpes, human cytomegalo, varicella zoster, vesicular stomatitis, yellow fever, bovine viral diarrhea, Semliki Forest, western equine encephalitis, encephalomyocarditis, type A rhino, respiratory syncytial, Newcastle disease, and type A influenza (H1N1). Neither IFN inhibited coxsackie B1, reo type 3 and vaccinia virus in the experimental conditions used. The specific activity of YM643 in human cells, ranged from $3.6x10^7$ to $2.1x10^9$ IU/mg, was greater than that of IFN-α (Namalwa), ranged from $3.1x10^6$ to $4.6x10^8$ IU/mg, except for HCMV and RV-1A. Of significance was the observation that the potency of YM643 against bovine diarrhea virus and yellow fever virus, which are selected to serve as surrogates of hepatitis C virus (HCV), equaled and exceeded that of IFN-α (Namalwa). These results suggest that the genetically engineered YM643 is more potent than a natural IFN-α.

28062

INDUCTION OF INDOLEAMINE 2,3 DIOXYGENASE IN HCV PATIENTS TREATED WITH INFERGEN.

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Interferon treatment often results in severe side effects including depression. The mechanism of induction of these side effects is unknown, but in part may be due to the induction of pro-inflammatory cytokines or other IFN-inducible genes. For example, the induction of indoleamine 2,3 dioxygenase (IDO) may result in decreases in tryptophan levels, which in turn may affect the levels of serotonin, known to be associated with depression. We have measured IDO activity in HCV patients (n=12) treated with infergen on a daily basis by measuring the levels of tryptophan and kynurenine in the plasma by HPLC at base line (before treatment) and during the course of treatment. Patients received 15 mcgs of daily infergen for 6 weeks, followed by randomization to 9 or 15 mcs twice a week.. Base line levels of kynurenine and tryptophan were within the normal range (kynurenine ave1.89 μmole, tryptophan av. 57.4μmole). Within 2 days of treatment the average kynurenine levels increased 2-3 fold. with a similar increase in kynurenine/tryptophan ratio. On an individual basis not all patients showed an increase in kynurenine, although a subgroup (7) did show an increase by day 2 following initiation of treatment and remained elevated throughout the treatment, although not at levels as high as on day 2. An increase in neopterin levels in some patients would suggest that this increase in IDO is through the induction of IFN-gamma. Beck Depression Inventory Score increased during the first 6 weeks of treatment. Thus it is possible that some of the side effects (or anti-viral effects) of interferon-alpha treatment are manifest through IDO and IFN-gamma induction.

28049

TNF-α INDUCES MATRIX METALLOPROTEINASE-9 GENE EXPRESSION AND SECRETION BY *MYCOBACTERIUM TUBERCULOSIS*-INFECTED HUMAN MONOCYTIC CELLS

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Mycobacterium tuberculosis (TB)-infected monocytic cells secrete matrix metalloproteinase-9 (MMP-9; 92kD gelatinase), which facilitates leukocyte recruitment to sites of infection but may cause tissue destruction, classically caseous necrosis. In order to investigate the MMP-9 secretion pattern *in vivo*, we first studied *M. tuberculous*-infected human lymph node specimens by immunohistochemistry. Strong MMP-9 staining was found in granuloma, principally located in monocytic cells and adjacent to caseous necrosis. We therefore investigated whether monocyte-monocyte cytokine networks might control secretion of MMP-9, measured by zymography, 52 kDa collagenase (MMP-1) or the specific tissue inhibitor of MMPs-1, (TIMP-1), both measured by ELISA. MMP-9 gene expression was also studied by northern analysis. Conditioned media from *M. tuberculosis*-infected monocytic cells (CoMTB) induced secretion of MMP-9 but not MMP-1 from uninfected, human monocytic THP-1 cells (183ng/ml at 4d; $p<0.05$ versus control). TIMP-1 was constitutively secreted and increased by CoMTB (134ng/ml at 4d; $p<0.05$). Pre-treatment of CoMTB with anti-TNF-α (100μg/ml) decreased MMP-9 secretion by 43% and TIMP-1 by 76% (both $p<0.05$). IL-1 receptor antagonist and pertussis toxin, which inhibits G-protein coupled processes stimulated by chemokines, had no effect. These data demonstrate that MMP-9 is present in monocytes in TB lesions associated with tissue destruction. TB-uninfected monocytes release MMP-9 due to cytokine networks and TNF-α, necessary for anti-mycobacterial granuloma formation, is critical in such monocyte-monocyte interactions.

28064

Induction Pattern of Cytokines Following a Single Injection of Infergen in Hepatitis C Patients

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Interferon-alpha is the major drug used in the treatment of hepatitis C. However the percentage of patients that show a sustained response (long term clearance of the virus and restoration of normal liver enzyme levels) is approximately 20%. We have initiated a systematic analysis of the response to interferon in a group of patients previously shown to be non-responders by examining serum samples taken every few hours following a single injection of IFN-con1 (Infergen). All such patients had antigenic type I HCV. The serum was examined for various cytokine levels and decrease in virus titer as measured by PCR.
In all patients (n=20), serum levels in IL-6 increased dramatically at 6-8 hours after treatment. This increase in IL-6 did not appear to be related to increasing dose of Infergen. There was no increase found in IL-2, IL-4, or TNF levels. In one patient there was an increase in IL-1β. Hepatitis C titers decreased (as measured by PCR) by a log or more within 24-48 hours of treatment, and then recovered to near base line by 72 hours. Thus injection with infergen stimulates pro-inflammatory cytokines in parallel with a decrease in virus titer. Similar results were found in a separate cohort of patients receiving continuous treatment with infergen. By 24 hours IL-6 levels had returned to normal or near normal values. Continuous exposure to interferon did not induce long lasting IL-6 production.

28030

Cell lines expressing Sendai virus C protein are insensitive to IFNs

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It has been reported that Sendai virus(SeV) infected cells were insensitive to IFN, and has been suggested that C proteins of the virus counteract IFN mediated antiviral state. Recently, the V protein of SV-5 was reported to be an inhibitor of IFN signaling targeting STAT-1 for proteasome-mediated degradation. Paramyxoviridae seem to have variable system to circumvent the IFN response, consequently persistent infections seems to be established. Here, we established HeLa cell lines expressing SeV-C protein, and examined IFN induced antiviral state and signaling including STAT-1, STAT-2 .
The C protein expressed Hela cells were completely insensitive to IFN activities even if the cells were treated with 10,000 IU of IFN-α,β,γ when VSV or EMCV were used as challenge viruses. IFN mediated signal transduction pathways in the C protein expressed cells were analyzed. Endogenous levesls of STAT-1 and STAT-2 compared with control HeLa cells were almost same, however, IFN induced increase of STAT-1 and PKR was prevented. The phosphorylation of the IFN related proteins are examined. In addition,Vero cells and L cells expressing SeV-C proteins were established and analyzed their properties. We recently found that HeLa cells expressing mumps virus V protein were IFN insensitive. We will discuss the mechanisms to circumvent paramyxoviruses from antiviral activities of IFNs.

28054

COMBINED ACTION OF INTERFERON AND dsRNA ENHANCES ANTIVIRAL EFFECTS

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In an attempt to enhance the antiviral effects of the IFN system on otherwise refractory viruses, we combined the action of two biological response modifiers ---IFN and dsRNA. Each reactant is known to induce IFN action genes independent of each other, and furthermore, dsRNA is required to activate at least two IFN action pathways. We postulated that the two reactants together might produce an antiviral effect greater than expected from strict additivity. We tested first a virus that is virtually totally refractory to IFN action, i.e., avian reovirus (ARV). rChIFN-α at 2000 U/ml for 24 hr had little or no effect on the efficiency of plaquing (EOP) of ARV on chicken embryo cells (CEC). A 50% reduction in EOP (PR_{50}) of ARV was achieved by dsRNA, in the form of pI:pC/deae-dextran, at 4,600 ng/ml dsRNA. When cells were pretreated with 2,000 U/ml IFN and then exposed to various concentrations of pI:pC, a PR_{50} value was obtained at 25 ng/ml. Thus, the combined sequential treatment of IFN and dsRNA resulted in a 184-fold enhancement in the antiviral effect. When Newcastle disease virus (NDV) was exposed only to IFN, about 50% of the virus population resisted its action over a wide range of doses (50-2,000 U/ml for 24 hr). Cells exposed only to pI:pC required ≈ 500 ng/ml pI:pC to achieve PR_{50}. Cells exposed to the combination of IFN (2,000 U/ml for 24 hr) and various concentrations of pI:pC achieved a PR_{50} value for NDV at 25 ng/ml pI:pC ---a 20-fold enhancement in the antiviral effect. How is the anti-antiviral effect intrinsic to these viruses overcome by the combined action of IFN and dsRNA? One model assumes that: (1) IFN pretreatment increases the level of dsRNA-dependent enzymes in the IFN action pathways, (2) exogenously added dsRNA exceeds the dsRNA-binding capacity intrinsic to the anti-IFN defense mechanism of the infecting virus, and thus, (3) sufficient free dsRNA is available to activate the IFN-mediated elevated levels of dsRNA-dependent enzymes in the IFN action pathways and to produce an enhanced antiviral effect.

28066

LEVELS OF IFN-γ, IL-12, IL-4 AND IL-10 ARE DETERMINED IN SERA AND SUPERNATANTS OF PBMC CULTURES FROM HYDATID PATIENTS.

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Human hydatidosis is parasitosis, which characterized by a prolonged coexistence of parasite, *Echinococcus granulosus* and host without effective rejection .The basis of the immune response of the patients begins to be understood. In fact, we reported previously a production of IFN, TNF-α, IL-6 activities in relation with immunoreactivity of patients against parasitic antigen (Antigen-5) (Touil-Boukoffa et al, 1997). In the present work, we investigate the involvement of Th1/Th2 balance in the mechanisms underlying seroreactivity in cystic echinococcosis. Th1 and Th2 cytokines were evaluated by enzyme- linked immunosorbent Kits (ELISA- Immunotech). IFN-γ, IL-12, IL-4, IL-10 production is determined in sera and in peripheral mononuclear cell (PBMC) cultures from patients (n=20) and healthy donors (n=10) stimulated by two soluble parasitic antigens (Antigen-5, PM=65 kDa and Fraction 4, PM=8 kDa) isolated from hydatic cyst fluid. The coexistence of elevated activities of IFN-γ, IL-12, IL-4, IL-10 is observed in most of sera from hydatid patients, which expressed positive serological reaction against the two antigens. These results support Th1 and Th2 cell activation in human hydatidosis. Th1 cells activation seems to relate to the Antigen-5 and Th2 cell activation to the Fraction-4.
Overall, These finding suggest that lymphocytes of subjects infected with *Echinoccocus granulosus* contains Th1 and Th2 like subpopulations.

28067

BACTERIAL POLYSACCHARIDES AS EFFECTIVE INDUCERS OF γ -INTERFERON PRODUCTION

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Search for inducers of the interferon endogenous synthesis was accomplished among the *Clavibacter michiganense* polysaccharides, differing by the composition and structure. It were explored the neutral polysaccharides (PS I), found to be the glucose homopolymers and acidic polysaccharides (PS II and PS III), identified as heteropolysaccharides, containing rhamnose, fucose and galactose in various molar ratios. In additional to the neutral monosaccharides their composition includes non sugar component, the pyruvic acid. Its presence is known to result in the stereochemical modification of the active functional sites and thus may effect the polysaccharide biological activity. Analysis of the interferoninducing activity made it possible to establish that neutral polysaccharides show insignificant activity (160-220 units) while interferoninducing activity is peculiar to acidic polysaccharides (850-2170 units). In order to estimate the putative role of the pyruvic acid within polysaccharides in the interferoninducing activity modified PS II and PS III were obtained. It was shown that polysaccharides lacking pyruvic acid failed to stimulate the interferon production. It seems probable the suggestion that pyruvic acid is responsible for the creation of such a conformation that would promote stimulating effect in interferon induction to assert itself. Through the inhibition of the interferon with the specific monoclonal antibodies to α – and γ –types of the human interferon it was shown that the polysaccharides inverstigated are the inducers of γ –interferon production.

28068

IL-1β COMPETES WITH CAPSULAR ANTIGEN CAF1 OF *YERSINIA PESTIS* FOR COMMON RECEPTORS ON IMMUNOCOMPETENT CELLS

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Specific receptors for recombinant human IL-1β (rHuIL-1β) (K_d=(3,1 ± 0,3)x10^{-10} M), rHuIL-1α (K_d=(2,1 ± 0,2)x 10^{-10} M) and for capsular antigen Caf1 of *Yersinia pestis* (K_d=(5,4 ± 0,1)x10^{-10} M) had been found on the cell surface of fibroblast finite cell line NIH 3T3. It should be noted, that Caf1 was capable of inhibition of high affinity binding of rHuIL-1β labeled with ^{125}I, but did not inhibit the binding of rHuIL-1α on of NIH 3T3 cells. In the presence of Caf1, specific binding of ^{125}I-rHuIL-1β is characterized by K_d=(2,0 ± 0,3)x10^{-9} M. rHuIL-1β and Caf1 stimulated ConA-induced mice thymocyte proliferation. According to the data obtained, Caf1 anchored on the bacterial sell surface could fulfil the role of *Y.pestis* adhesin. The interaction of Caf1 with huIL-1 receptor could play an important role in the fatal human pneumonic plague pathogenesis. Capsule formation in *Y. pestis* was induced at 37^0 C. *In vivo* that process occurred in human lungs after aerosol infection. Newly expressed Caf1 could closely attach the bacteria to lung immunocompetent cells via Caf1-IL-1 receptor interaction and hence induce the expression of the type III secretion system.

Presenting author: Vasiliev A.,
Poster presentation,
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28069

Immunohistochemical detection of the interferon induced PKR in liver biopsies from patients chronic viral hepatitis B and C

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The interferon induced ds RNA activated protein kinase, PKR, mediates the antiviral effects of interferon. We studied the expression of PKR in routinely processed liver biopsies using monoclonal PKR Ab (kindly provided by Dr. A. Hovanessian, Institut Pasteur, Paris) in liver biopsies(LB) from 25 patients with chronic hepatitis B(all HBsAg+, HBcAg+ HBV-DNA+) and chronic hepatitis C(all anti-HCV+, HCV-RNA +). LB from patients with minimal change liver disease and primary biliary cirrhosis(PBC-all AMA +) were used as controls.

Disease – patient #	sex (m/f)	age (mean +/- SD)	ALT (mean +/- SD)
minimal change-7	5/2	41+/-10	20+/-16
HCV - 11	7/4	34+/-17	59+/-31
HBV - 14	10/4	38+/-10	80+/-46
PBC – 14	2/12	50+/-11	84+/-41

PKR visualization required antigen retrieval in 0.1mmol citrate buffer, pH 6.0, over night incubation at RT, and DAKO LSAB2 DAB kit. The results are shown on the following table:

Disease - patient #	Hepatocytes - Cytoplasm expression score * (mean +/- SD)	hepatocytes - nuclear expression # of +biopsies	biliary cells cytoplasm expression # of +biopsies	sinusoidal cells - cytoplasm expression # of +biopsies
minimal change	1.57+/-1.27	0	1	2
HCV	2.18+/-1.7**	8 (p=.012)***	8 (p=.049)***	1
HBV	2.5+/-1.34**	5	3	4
PBC	2.4+/-1.55**	3	1	0

*score: 1=1-25%, 2=26-50%, 3=51-75%, 4= 76-100% of the hepatocytes. ** not significant - Mann-Whitney U test. ***Fisher exact p

We conclude that during hepatitis C the number of PKR expressing biliary cells increase and PKR appears in the hepatocyte nuclei.

28070

Different *Leishmania* species induce distinct profile of produced cytokines in murine macrophages

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CBA mice are resistant to *L. major* (*Lm*) but susceptible to *L. amazonensis* (*La*) infection. In murine leishmaniasis the early events of infection are crucial to disease outcome and macrophages (MΦ) play a central role in these events as they are the cells that harbor parasites and can present antigens to specific T lymphocytes. MΦ phagocytizes *Leishmania* promastigotes and once activated can destroy parasites by a NO-dependent killing mechanism. In this report we evaluated the differential capacity of CBA peritoneal MΦ to destroy *in vitro Lm* or *La*. We determined the % of infected cells and the n.° of parasites per infected MΦ activated or not with rIFN-γ after different periods of infection. We determined NO production by Griess reaction and iNOS mRNA levels by RT-PCR to assess IFN-γ capacity to activate *Lm*- or *La*-infected MΦ. The ability of infected cells to produce cytokines that modulate MΦ activation was assessed by the determination of IL-10 and TGF-β mRNA expression by RT-PCR. Our results showed that between 90 min and 12 hours after addition of promastigotes, the proportion of infected MΦ and the n.° of parasites per MΦ were similar between *Lm*- and *La*-infected MΦ. Interestingly, 36 hours after infection, the percentage of *La* infected MΦ was almost 2.0 times higher in comparison to *Lm*-infected cells. These differences were maintained during the next 60 to 84 hours. At these time points, the n.° of parasites per cell was 2.0 times higher in *La*-infected cells. In addition, at least between 12 and 36 hours of infection, in both *Lm*- or *La*-infected cells, IFN-γ did not modify neither the % of infected MΦ, nor the n.° of parasites per cell, despite iNOS mRNA expression and NO production were similar by *Lm*- and *La*-infected cells. In regard to cytokine production non activated *La*-infected MΦ showed 2 times higher TGF-β mRNA levels than *Lm*-infected cells. However, IL-10 mRNA was enhanced 86h after infection similarly in both non activated *Lm*- and *La*-infected cells. When MΦ were pretreated with IFN-γ, only in *Lm*-infected cells, TGF-β and IL-10 mRNA expression were downregulated. These data showed that only in La-infected MΦ cytokines that balance towards Th-2 response are not modulated after IFN-γ activation. Works are in progress in order to determine mRNA expression by *Lm*- or *La*-infected MΦ of cytokines that balance immune response towards Th1 response such as IL-12p40 and TNF-α. These data suggest that *Lm* and *La* are capable to differentially affect MΦ effector functions *in vitro* and that it could be important to determine resistance or susceptibility to *in vivo Leishmania* infection.

28071

DIFFERENTIAL REGULATION OF CHEMOKINE SECRETION BY T_H2 CYTOKINES, DEXAMETHASONE AND PGE_2 IN TUBERCULOUS AND STAPHYLOCOCCAL OSTEOMYELITIS

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Osteomyelitis (OM), or bone infection, is an important cause of morbidity worldwide and is characterised by loss of bone structure as a result of bacterially-induced inflammation. Previous work in this laboratory has shown that infection of the human osteoblastic cell line, MG-63, with *Mycobacterium tuberculosis* (MTB) or *Staphylococcus aureus* (SA), major pathogens causing OM, induces production of C-X-C and C-C chemokines. Using ELISAs to study secretion and a biotinylated RNase Protection Assay to assess gene expression we investigated the effects of T_h2-derived cytokines (IL-4, IL-10 and IL-13), dexamethasone and PGE_2 on chemokine secretion. In MTB-infected cells, IL-4 increased IP-10 (from 12033pg/ml ±1069 to 24333 pg/ml ±1761), RANTES (3552pg/ml ±152 to 6926pg/ml ±400) and MCP-1 (89700pg/ml ±12947 to 115666pg/ml ±6393) secretion but had no effect on IL-8. This contrasted with the downregulatory effects of IL-10 and IL-13 which reduced chemokine secretion to near control levels. SA-induced chemokine secretion was similarly affected. IL-4 increased the secretion of IP-10 (from 15916pg/ml ±299 to 55700pg/ml ±1388), RANTES (642pg/ml ±43 to 1401pg/ml ±145) and MCP-1 (12679pg/ml ±1744 to 55700pg/ml ±1388) but not IL-8. However, unlike in MTB infection, IL-10 and IL-13 also increased RANTES and MCP-1 secretion. Concurrent changes in mRNA expression were observed. Dexamethasone and PGE_2 were potent mediators of chemokine down-regulation reducing levels of chemokine secretion and gene expression almost to control unstimulated levels at concentrations as low at 10^{-8}M. In conclusion, these data show that chemokine secretion is differentially regulated by T_h2 cytokines in MTB- and SA-infected osteoblastic cells. In contrast, chemokine secretion is uniformly down-regulated by dexamethasone and PGE_2.

28072

ROLE OF IFN-γ AND TNF-α IN H. PYLORI INFECTION

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The immune responses to *Helicobacter pylori* (*H. pylori*) infection play important roles in gastroduodenal diseases. The contribution of interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) to the immune responses, especially to the induction of gastric inflammation and to the protection from *H.pylori* infection, was investigated with IFN-γ gene-knockout (IFN-γKO) and TNF-α gene-knockout (TNF-αKO) mice. We first examined the colonizing ability of *H. pylori* strain CPY2052 in C57BL/6 wild-type and KO mouse stomach. The number of *H. pylori* colonized in the stomach in IFN-γKO and TNF-αKO mice was higher than that in wild-type mice. These findings suggested that IFN-γ and TNF-α may play a protective role in *H. pylori* infection, although the degree of its protective ability was estimated to be low. Furthermore, we examined the contribution of IFN-γ and TNF-α to gastric inflammation. The CPY2052-infected TNF-αKO mice developed a modelate infiltration of mononuclear cells in the lamina propria and erosions in the gastric epithelium as well as wild-type mice, whereas the CPY2052-infected IFN-γKO mice showed no inflammatory symptoms 6 months after infection. These results clearly demonstrated that IFN-γ but not TNF-α play an important role in the induction of gastric inflammation caused by *H. pylori* infection.

28073

MOLECULAR MECHANISM OF DEADLY PROINFLAMMATORY RESPONSE IN PATHOGENESIS OF FATAL PNEUMONIC PLAGUE

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Yersinia pestis, the causative agent of human fatal pneumonic plague, provokes the death of host in a result of septic shock in a few days after aerosol infection. Here we suggest the mechanism of deadly proinflammatory response to the infection. *Y. pestis* has in its arsenal two clusters of genes belonged to the class of operons responsible for assembly of adhesive structures on the surface of pathogenic Gram-negative bacteria via chaperone-usher pathway. These operons, *caf* and *psa*, are responsible for expression of capsular F1 and pH6 antigens (Ag) correspondingly. We discovered that low molecular weight (LMW) forms of Caf1 bind interleukins-1 (ILs-1). It was found that PsaA protein is IgG-binding receptor displaying reactivity with human IgG1, IgG2 and IgG3 via the Fc fragments. It was also discovered that PsaA protein causes activation of tumor necrosis factor α (TNFα) synthesis in macrophage-like HL-60 cells. The results obtained allow to suggest that newly expressed at 37°C after aerosol infection LMW forms of Caf1, can closely attach bacteria to lung macrophages via Caf1-proIL-1 interaction. The expressed after acidification of extracellular medium by macrophages pH6 (PsaA) antigen adheres *Y. pestis* to the cells via PsaA-IgG-FcγR1 complex formation and triggers off TNFα expression that may be a cause of septic shock and death of host. Therefore new methods of therapy of plague require new generation of medicines neutralizing deadly proinflammatory response of macrophages/monocytes.

28074

MULTINUCLEATED GIANT CELLS (MGC) AND THE CONTROL OF CHEMOKINE SECRETION IN TUBERCULOSIS

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MGC are a characteristic feature of tuberculous granulomas, but the mechanisms that regulate their formation and function are not presently understood. The aims of our study to investigate CC and CXC chemokine secretion (i) during and after MGC formation (ii) from MGC infected by mycobacterium tuberculosis (TB) (iii) from monocytes exposed to conditioned medium from MGC. MGC were generated *in vitro* by stimulation of human peripheral blood monocytes with ConA (5μg/ml) and IFN-γ (1000IU/ml) for 3 days, and fusion rates of up to 80% were obtained. Chemokine gene expression was assessed by non-isotopic RNase protection assays and secretion by ELISA. During the course of MGC formation (72hrs), increasing concentrations of IL-8 (up to 12+/-0.2ng/ml), MCP-1 (up to 15+/-0.5ng/ml) and IP-10 (up to 4+/-0.2ng/ml) were found in the supernatant. However, after MGC were formed, unstimulated MGC only released low levels of chemokine, and infection with TB did not increase this. Interestingly, although MGC conditioned medium alone upregulated secretions of these chemokines from monocytes, addition of TB with MGC conditioned medium decreased levels of all three chemokines in the supernatant (decreased up to 50%). In summary, MGC formation results in secretion of chemokines. Once established, MGC secreted only low levels of chemokine. Addition of MGC conditioned medium with TB downregulated chemokine secretion from monocytes. The mechanisms underlying these data are currently being investigated.

28075

ACTIVATION OF CYTOKINE PRODUCTION BY ANTIVIRAL ACYCLIC NUCLEOSIDE PHOSPHONATES

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Several 9-[2-(phosphonomethoxy)ethyl] (PME) and 9-[2-(phosphonomethoxy)propyl] (PMP) derivatives of purine bases (adenine [A], guanine [G], diaminopurine [DAP]) and cytosine [C]) exhibit potent activity against a great variety of both DNA viruses and retroviruses, including HIV and hepatitis B virus. The postulated mechanism of antiviral action of acyclic nucleoside phosphonates (ANPs), is inhibition of replicative DNA-polymerases. In the present experiments we have investigated possible immunomodulatory properties of ANPs, namely their interference with secretion of cytokines. The effects were screened mostly in an *in vitro* murine cell system, using resident peritoneal macrophages and splenocytes. Some compounds such as PMPA (*Tenofovir*), (R)-PMPDAP, and PMEG have been found to stimulate in a dose-dependent manner the secretion of TNF-α, IL-10, and chemokines RANTES and MIP-1α. Production of IL-12 and IL-18 remained uninfluenced. The enhanced RANTES expression has also been observed in the human *in vitro* cell systems. In addition, these ANPs have proved to up-regulate the IFN-γ-triggered synthesis of nitric oxide (NO) by mouse macrophages. It can be concluded that some acyclic nucleoside phosphonates possess prominent immunobiological activities which may be of practical significance in immunotherapy of viral infections.
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28063

INTERLEUKIN 1 AND TUMOR NECROSIS FACTOR α AUGMENT THE MYCOBACTERIA INDUCED HOST CELL DEATH

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In this study we showed proinflammatory cytokines, interleukin 1 (IL-1) and tumor necrosis factor α (TNF-α) augment the cytotoxic effect of virulent mycobacteria on human lung fibroblast cell line, MRC-5. When MRC-5 was infected with various strains of mycobacteria (*M. tuberculosis* $H_{37}Rv$ and $H_{37}Ra$, *M. avium* 427S and 2151SmO, and *M. bovis* BCG Pasteur and Tokyo), the fibroblasts were killed by mycobacteria according to the degree of virulence. Other human originated macrophage (U937, THP-1), myeloid (HL-60) and epithelial carcinoma (A549) cell lines also exhibited similar cytotoxic response to virulent mycobacteria. MRC-5 was most susceptible to virulent mycobacteria among various human cell lines examined. IL-1 and tumor TNF-α promote the growth of MRC-5 fibroblast in dose dependent manner. But it was found that when the cells were infected by virulent bacilli, these cytokines augment the bacterial cytotoxicity. An inhibitor of interleukin 1β converting enzyme partially inhibited the bacterial cytotoxic effect on the fibroblast. These results indicate that mycobacteria infection modulate the susceptibility of fibroblasts to IL-1 and TNF-α.

28038

MICROBIAL INFECTION INDUCES TLR GENE EXPRESSION

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Toll-like receptors (TLRs) have been shown to mediate innate immune responses to bacteria and other microbes. TLRs sort out microbial antigens, and upon bacterial stimulation induce NF-κB activation. TLR2 has been implicated in responses to various microbes including Gram-positive bacteria, while TLR4 appears to be specific to Gram-negative bacteria. We have analyzed the induction of TLR1- 6 in human primary macrophages as well as in epithelial and endothelial cells by Gram-positive bacteria *Lactobacillus rhamnosus and Streptococcus pyogenes*, and compared responses to those induced by viruses. Our results show that in macrophages TLR2 was induced by both Gram-positive bacteria and viruses, while TLR3 was induced by *S. pyogenes* and viruses. TLR3 induction was IFN-α-inducible with purified IFN-α inducing strong TLR3 expression, and bacteria-induced TLR3 expression was inhibited by neutralizing antibodies to IFN-α/β. TLR3 was also induced in epithelial and endothelial cells by viruses and purified IFN-α but not by bacteria.

28031

GENE THERAPY OF AIDS BY CONSTITUTIVE EXPRESSION OF INTERFERON β : EXPERIMENTAL APPROACH IN MACAQUES CHRONICALLY INFECTED WITH A PATHOGENIC PRIMARY ISOLATE OF SIV_{mac251}.

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The efficacy of a gene therapy strategy of HIV infection, based on the constitutive and low expression of interferon β, was assessed in macaques infected with a pathogenic SIVmac251.
Asymptomatic cynomolgus macaques, infected for more than one year with a pathogenic primary isolate of SIVmac251, were included in this experiment. Before the experiment, all animals exhibited low plasma and cell-associated viral loads and their circulating $CD4^+$ T-lymphocytes were higher than 700 cells/µl.
Retroviral vector used derives from MoMuLV and allows the constitutive expression of macaque interferon β. Con A activated monkey PBL were transduced *ex vivo* then reinfused into macaques. Three groups of three animals received either cells expressing the transduced Interferon β gene, a deleted construct or both kind of transduced cells. Virological and immunological parameters of SIVmac251 infection were followed up.
After reinfusion of autologous transduced cells in macaques, copy numbers of the therapeutic gene varried between 70 and 1000. per 10^6 cells. Few days after reinfusion, a transient increase of cellular and plasma viremia was noticed in most of the animals, regardless the retroviral construct received. Long-term follow-up indicate that injection of cells with the vector expressing interferon β, did not protect macaques from the progressive CD4+ lymphocyte decrease which characterizes SIV infection.
Injection of transduced cells may be accompanied by transient adverse effects. Low percentages of circulating interferon beta-gene transduced cells did not prevent macaques from progression to disease.

28032

TYPE I INTERFERON SUBTYPES PRODUCED BY HUMAN PERIPHERAL MONONUCLEAR CELLS STIMULATED BY VARIOUS INDUCERS.

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Viral infections stimulate the transcription of interferon (IFN) type I, which includes IFN-α (13 subtypes) and IFN-β (a single molecule). Mechanisms of type I IFN production by human peripheral blood mononuclear cells (PBMCs) are not well understood. The aim of this study was to identify the type I IFN subtypes produced *in vitro* by human PBMCs. These cells, obtained from a single donor, were exposed to various agents (Sendai virus, Herpes simplex virus type 1 , poliovirus-IgG complexes and a serum from a patient with systemic lupus erythematosus). Six hours after the addition of the inducing agent, RNA was extracted, treated by DNAse and transformed into cDNA by a reverse-transcription reaction with random hexamers. Then, the cDNA was amplified by PCR using primer pairs which recognize either IFN-A (all the subtypes) or IFN-B or G3PDH (as internal control) genes. The overexpression of IFN-α and IFN-β mRNA was observed with each inducing agent. The identification of IFN-α subtypes was performed by cloning and sequencing of the 375 bp amplification products corresponding to IFN-α PCR. The most highly abundant subtypes were: A4 and A5 (constitutive expression), A21 (lupus), A5, A8 and A17 (virus Sendai), A5 and A17 (poliovirus-IgG complexes), A5, A10 and A21 (Herpes simplex virus). Thus, the relative abundance of some IFN-α subtypes appears to depend on the inducer. The implications of parameters such as genetic factors of the donor and the non-purified nature of PBMC need further characterization. The mechanisms by which somes IFN-α subtypes are preferentially expressed, and the consequences of such a phenomenon will be very important to elucidate.

28033

ASTROCYTIC EXPRESSION OF MMP-2 AND MMP-9 IN RESPONSE TO HIV AND TNFα.

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HIV causes frequent neurological complications. Because of their localisation at the blood-brain barrier, astrocytes are among the first cells in contact with HIV in the central nervous system. Matrix metalloproteinases are implicated in the blood-brain barrier remodelling and integrity. In this context we examined whether HIV could act on MMP and TIMP expression by astrocytes. We used three human glioblastoma cell lines (CB193, T98G and U118MG) and simian primary astrocytes. Cells were stimulated with semi purified heat-inactivated HIV-1/Lai (1, 10 and 100 ng/ml of p24) and co-stimulated with TNFα at 10 and 100 ng/ml. We used a medium without FCS to avoid FCS-originating MMP contamination. Cells were stimulated 3 days and supernatants were used for gelatin zymography analysis and TIMP-1 and TIMP-2 ELISA (Amersham). HIV particles enhanced MMP-2 expression, and TNFα strongly increased MMP-9 expression in astrocytes. HIV did not affect TIMP-1 or TIMP-2 production whereas TNFα up-regulated TIMP-2. By degrading collagen IV and laminis of basal membranes, this up-regulation of MMP-2 by HIV may cause a blood-brain barrier disruption which would promote the recruitement of infected cells through the blood brain barrier. In response to TNFα, produced by infected macrophages, astrocytes could then enhance this phenomenon through MMP-9 release. These results show that, in an inflammatory context of HIV infected CNS, astrocytes are able to participate to the penetration by infected monocytes/macrophages by producing matrix metalloproteinases which could impair the blood brain barrier integrity.

28034

INFLUENCE OF RECOMBINANT INTERFERON α2b IN GEL (VIFERON-GEL®) ON INTERFERON STATUS IN CHILDREN WITH OFTEN AND PROLONGED DISEASES

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Efficiency of the Viferon-gel® treatment was studied in 24 children 1 to 3 years old – 5 with stenosing laringotrachcitis (**SLT**), 5 with recidivating obstructive bronchitis (**ROB**) and 14 with often and prolonged diseases (**OPD**). Interferon status was determined according to S.S. Grigoryan (1988). Level of activity of natural killers – activated NK (CD 16+8+) was determined by method of membrane immunofluorescence. Viferon-gel® (human recombinant interferon $_{\alpha 2\beta}$ in the form of gel, 40 000 IU/ml with antioxidants) was applied to orolaryngeal mucosa three times daily for one month with hard tampon. Before Viferon-gel® therapy α-IFN level was 16.8±1.2, 13.8±2, 8.6±1.2, for **SLT**, **ROB** and **OPD** accordingly (17.6±1.3 in norm). γ-IFN level was **SLT**: 4.54±0.9, **ROB**: 6.1±0.2, **OPD**: 6.1±1.6 (15.4±1.2 in norm). Serum IFN level was **SLT**: 1.8±0.2, **ROB** 2.8±0.1, **OPD** 4.3±0.5 (6.2±0.1 in norm). Level of activity of natural killers – activated NK was **SLT**: 1.2±0.3, **ROB:** 0.8±0.03, **OPD:** 1.1±0.1 (1.4±0.2 in norm). After Viferon-gel® treatment α-IFN level was 19.5±1.5, 19.6±1.4, 18.5±0.9, for **SLT**, **ROB** and **OPD** accordingly (17.6±1.3 in norm). γ-IFN level was **SLT**: 8.8±1.1, **ROB**: 7.9±0.1, **OPD**: 9.4±0.8 (15.4±1.2 in norm). Serum IFN level was **SLT**: 2.8±0.1, **ROB:** 3.0±0.6, **OPD:** 4.8±0.3 (6.2±0.1 in norm). Level of activity of natural killers – activated NK was **SLT**: 0.8±0.5, **ROB:** 2.5±0.1, **OPD:** 3.3±0.06 (1.4±0.2 in norm). Thus increased sensitivity to infectious diseases in children with **SLT**, **ROB** and **OPD** is associated with imperfect functioning of the interferon system, which requires therapy by interferon combined with antioxidant correction, in particular by Viferon-gel®.

28035

RECOMBINANT INTERFERON α2β (VIFERON-GEL®) IN TREATMENT OF HERPES SIMPLEX INFECTION

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The aim of the study was to investigate the effect of viferon-gel which include human α2 interferon recombinant and antioxidants in complex treatment of recurrent herpes simplex.
Study methods:
30 patients were included in study of interferon status in the blood serum and treated topical by viferon-gel. We used immunoassay method for diagnostics of HSV1 and HSV2 types.
Results:
We established decrease of α and γ-interferon more, than twice, and also increase of level IgG in the blood serum. The decrease of life quality was marked. After complex treatment by viferon-gel and rectal suppositoria, the parameters of the interferon status have achieved normal meanings. The antibodies Ig G to HSV1 type in the blood serum were absent, and to HSV type 2 remained within the limits of low meanings.
Conclusion: The local treatment with viferon –gel 40 ug and rectal suppositoria 500000 ug was found to have a significant effect in patients with recurrent HSV infection. The subjective attributes disappeared on 2 days, and complete health came on 5 days after applications by viferon-gel. The life quality high was marked.

28056

CYTOKINES AND SOME IMMUNOLOGIC PARAMETERS IN PATIENTS WITH DIFFERENT VARIANTS OF SID IN THE DYNAMICS OF IMMUNOACTIVE THERAPY.

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Wide spreading of secondary immunodefitiency (SID) in patients with various infections and inflammatory diseases as well as difficulty in therapy makes an actual problem to study of its formation mechanisms in two pathologic variants: chronic bacterial infection, such as chronic bronchitis (CB) and chronic viral – such as relapsing herpetic infection (RHI). The purpose of our research was to study the subpopulation structure of immunocompetent peripheral blood cells, their functional properties as well as the serum levels of the cytokines in patients with CB and RHI in dynamics of immunoactive therapy by polyoxidonium and viferon (rh α2-interferon) respectively. In the studied groups there were significant differences in some parameters of the immune status in comparison with healthy donors. RHI patients had an increase in the content of main subpopulations of T-cells and decrease in the activity of DTH effectors and monocytes. In CB the number of CD8+ cells was increased while the CD16+ and the activity of DTH effectors were decreased. As for blood serum cytokines contains, it was found to have the reliable and significant distinctions from corresponding indices in donors (10-100-fold more).CB group in comparison with RHI was determined to prevail of significant increasing of TNFα level (2026 and 315pg/ml respectively), IL-2 (6835 and 1403pg/ml) and IL-6 (1007 and 38 pg/ml). On the contrary, IL-4 has been demonstrated 4,5 times less levels in CB patients than in RHI ones (58 and 265 pg/ml). In CB patients had been treated by polyoxidonium the contents of all measured cytokines were reliable decreased, including significant decreasing of TNFα, IL-2 and IL-6 levels. However they were remained more than in norm. In RHI patients had been treated by viferon took place a decreasing of TNFα and IL-6 levels and significant decreasing of IL-4 level (became lower than in norm). But content of IL-2 had been some increasing, that was correlated with enhancing of spontaneous and ConA stimulated PB MNC proliferatory activity. Aside from, in the dynamics of used therapy in both groups there is a tendency towards normalization of some changed indices of the immune status. The role of studied parameters in SID formation and optimization of therapy is being discussed.

28037

DETERMINATION OF POSSIBLE INFLUENCE OF IFN PREPARATIONS AND IFN INDUCERS ON CYTOKINES' PRODUCTION IN PATIENTS WITH VIRAL INFECTIONS

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Proinflammatory and antiinflammmatory cytokines play an important role in pathogenesis of virus diseases. The drugs, which increase antiinflammmatory cytokines' production and decrease the proinflammatory one, would be useful for treatment of viral infections. We proposed to determine possible influence of IFN preparations and IFN inducers on cytokines' production in whole blood cell probes obtained from the patients before treatment.

5 patients with hepatitis C (HCV), 9 patients with herpes virus infection (HSV-2) and 5 healthy volunteers were studied. IL-1β, IL-4, IL-6, IL-8, TNF-α and IFN-α presence in whole blood probes was determined by ELISA after their induction by IFN-α, IFN-γ or IFN inducers Ridostin, Cycloferon, Kagocel, Phosprenyl.

It was shown that only IFN-γ and Ridostin increased the antiinflammatory cytokine (IL-4) production in whole blood cells of volunteers and patients with HCV. On the contrary, IFN-α decreased it in cells of volunteers. Also we have found that some of IFN preparation and IFN inducers decreased proinflammatory cytokines' production by whole blood cells of patients with HCV and HSV-2. Production of IL-6 was decreased by Cycloferon, IFN-α and IFN-γ. Production of IL-8 was decreased by Cycloferon and IFN-γ. Production of TNF-α was decreased by IFN-α and IFN-γ.

We suppose that these drugs may be used in the treatment for regulation of own cytokines' production by the cells of patients with viral diseases.

28023

INTERFERON-γ AND INTERLEUKIN-10 INHIBIT MATRIX METALLOPROTEINASE-9 SECRETION FROM *MYCOBACTERIUM TUBERCULOSIS*-INFECTED HUMAN MONOCYTIC CELLS.

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The balance between Th1 and Th2 derived cytokines regulates the immune response to *Mycobacterium tuberculosis* and the extent of host tissue destruction. Unrestricted matrix metalloproteinase-9 (MMP-9) secretion is associated with tissue injury *in vivo*. We therefore investigated effects of Th1 and Th2 cytokines on MMP-9 gene transcription (by northern analysis) and secretion (by zymography) in human monocytic cells, pivotal in anti-mycobacterial immunity. In addition, tissue inhibitor of MMP-9 (TIMP-1) concentrations were measured by ELISA; TIMP-1 is the principal monocyte-derived MMP-9 inhibitor. After pre-incubation with IFN-γ, secretion of MMP-9 at 48h from human monocytic THP-1 cells infected by live, virulent *M. tuberculosis* (H37Rv) was reduced from 160ng/ml to 50ng/ml ($p<0.05$). Pre-incubation with IL-10 (100ng/ml) reduced MMP-9 secretion by 25% in monocytic cells infected with *M. tuberculosis*, although completely abolished secretion after LPS-stimulation ($p<0.05$). TIMP-1 is constitutively secreted by control cells (concentration at 48h = 25ng/ml) but did not alter after infection with *M. tuberculosis*, or pre-incubation with IL-10 (100ng/ml). IL-4 and IL-13 did not affect MMP-9 secretion. In conclusion, IFN-γ and to a lesser extent IL-10 (but not other Th2 cytokines) down-regulate MMP-9 secretion by monocytic cells. IFN-γ, critical in anti-mycobacterial immunity, may have a novel role inhibiting MMP-9-mediated tissue injury in tuberculosis.

28039

Role of Interferon-γ and Tumor Necrosis Factor-α in Herpes Simplex Virus Type 1 infection

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Herpes simplex virus type 1 (HSV-1) has the characteristics of acute and latent infection, and reactivation from latency. Cytokines have been proposed to play an important role in each state of HSV-1 infection, but the exact role of cytokines still remains unclear. In the present study, we investigated the role of IFN-γ and TNF-α in acute infection and reactivation by using IFN-γ gene knockout (GKO) and TNF-α gene knockout (AKO) mice. We first examined the survival rate after corneal infection with 1.5 X 10^6PFU of HSV-1 (Amakata strain). The survival rates of C57BL/6 wild-type, GKO and AKO mice were 97% (73/75), 57% (24/42) and 83% (60/72), respectively. These results indicate that TNF-α as well as IFN-γ play a protective role in HSV-1 acute infection. Furthermore, we examined the rate of reactivation induced by ultraviolet light in latently infected mice over 60 days post-infection. The latent infection and reactivation were confirmed by detection of viral DNA extracted from eye ball (EB) and trigeminal ganglion (TG) by PCR. The rate of reactivation in GKO (50%) and AKO (45%) mice were significantly higher than that in wild-type (16%) mice. These results suggest that IFN-γ and TNF-α play an important role in reactivation from latency.

28040

IFN-γ, IFN-γ RECEPTOR, IFN-γ mRNA, AND STAT1 mRNA IN HUMAN APPENDIX

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Many appendixes removed from patients with suspected acute appendicitis are subsequently classified as normal (ranges: 7.7-54%) by conventional histological staining. Are such histologically normal appendixes always normal at a molecular level? A substantial proportion showed clear evidence of an inflammatory response in the form of increased TNF-α and IL-2 mRNA expression (Y Wang et al. *Lancet* 347: 1076, 1996). To extend this study to other cytokine, we examined the expression of IFN-γ, IFN γ receptor (IFN-γR), IFN-γ mRNA, and STAT1 mRNA in histologically normal appendixes, using immunohistochemistry and an *in situ* hybridization (ISH) method. Appendix specimens were formalin fixed, paraffin embedded and sections (7μm) were stained with H&E, for histopathological analysis, or treated with Envision plus peroxidase/alkaline phosphatase method (Dako) for immunoenzimatic technique, using appropriate monoclonal antibodies. To detect IFN-γ mRNA and STAT1 mRNA we used a sensitive, non-radioactive ISH method. The probes for IFN-γ and STAT1 were obtained from human lymphocytes with RT-PCR and digoxigenin (Dig)-labelled in a repeated PCR and the hybridization was developed with sheep antibodies (AP-lebeled) directed against Dig. Twenty per cent of histologically normal appendixes showed large numbers of IFN-γ$^+$ cells in the lamina propria among the glandulae, some positive cells penetrating the epithelial and muscular layers, and some cells into the appendix lumen. Sometimes, intense accumulation of IFN-γ was present extra-cellularly in the extrafollicular area, suggesting that IFN-γ is associated with extracellular matrix components. To define whether IFN-γ$^+$ cells are producing IFN-γ or taking it up from surrounding tissue we used ISH method. IFN-γ mRNA and IFN-γR staining were found with the same distribution of IFN-γ. STAT1 mRNA$^+$ cells had the same distribution of IFN-γR$^+$ cells. We suggest that local expression of IFN-γ by inflammatory cells could be, at molecular level, a sign of focus inflammation in those appendixes that microscopically appear normal and IFN-γ could be implicated, with other cyokines such as TNF-α and IL-2, in pathogenesis of appendicitis.

28041

NON-LPS COMPONENTS OF *CHLAMYDIA PNEUMONIAE* STIMULATE SYNTHESIS OF CYTOKINES INDEPENDENTLY OF TLR4

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It has been recently suggested that infection with *Chlamydia pneumoniae* is associated with atherosclerosis, and that cytokines play an important role in the intiation and progression of *Chlamydia*-induced inflammation. The pathways responsible for the stimulation of cytokines by chlamydial components have been investigated. Stimulation of resident mouse peritoneal macrophages for 24h with sonicated *C. pneumoniae* (10^4 inclusion forming units/ml), induced the synthesis of significant amounts of tumor necrosis factor-α (TNF), interleukin-1α and β (IL-1) and IL-6. Macrophages harvested from TNF and lymphotoxin-α (TNF-/-LT-/-) deficient mice synthesized significantly less IL-6 (64% inhibition, $p<0.02$) compared to controls, suggesting that TNF and LT have an important role in the activation of second-wave cytokines induced by *C. pneumoniae*. In contrast, no effect of TNF and/or LT on IL-1α and IL-1β production after challenge with *C. pneumoniae* was apparent. Heat-inactivation of chlamydial components resulted in a 57% ($p<0.05$) reduction of IL-1α and 82% ($p<0.01$) reduction of IL-6 synthesis, suggesting that a heat labile non-LPS component, and not the heat-resistant LPS, is responsible for the cytokine activation. Neutralization of endotoxin using polymyxin B also revealed that chlamydial LPS plays a minor role in the cytokine induction. In addition, normal induction of cytokines by *C. pneumoniae* in C3H/HeJ mice (TNF: 267 +/- 33 pg/ml in C3H/HeJ vs. 224 +/- 41 pg/ml in control C3H/HeN mice, $P>0.05$) demonstrate that the non-LPS components responsible for the stimulation of cytokine production act in a TLR-4 independent manner. In conclusion, non-LPS components of *C. pneumoniae* are a potent stimulus for cytokine production acting independently of TLR-4, and these findings may be important for the pathogenesis of atherogenesis.

28042

DIFFERENTIAL REGULATION OF TOLL-LIKE RECEPTOR (TLR) 2 AND TLR4 GENE EXPRESSION IN MURINE LIVER AND HEPATOCYTES BY ENDOTOXIN AND CYTOKINES

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We have previously shown that type I IL-1receptor (IL-1RI) mRNA expression in the liver was up-regulated by administration of endotoxin (LPS), IL-1, IL-6 or TNF into mice and that IL-1, IL-6 and glucocorticoid mediated the up-regulation. As Toll-like receptor (TLR) 2 and TLR4 are members of IL-1R family and transduce similar signals as IL-1RI in response to bacteria and bacterial components, it is interesting to investigate whether LPS and cytokines regulate their gene expression in a similar manner. When mice were administered with LPS, TLR2 mRNA was up-regulated in the brain, heart, lung, liver and kidney. In contrast, it was down-regulated in the spleen. TLR4 mRNA was decreased in the brain. In the heart and lung, it increased, whereas it was not affected in the liver, kidney and spleen. TLRs mRNA was further analyzed in the liver and hepatocytes. Like LPS treatment, administration of IL-1, IL-6 or TNF, all up-regulated TLR2 mRNA. However, none of them affected TLR4 mRNA level. In primary cultured hepatocytes TLR2 mRNA was up-regulated by LPS, IL-1 or TNF, but not by IL-6 or dexamethasone. None of them affected TLR4 mRNA expression. Similar responses were observed in murine hepatoma cell line Hepa 1-6. These results suggest that in infection with Gram-negative bacteria, LPS and proinflammatory cytokines differentially regulate gene expression of TLR2, TLR4 and IL-1RI in murine hepatocytes, which may lead to pathological and host defense reactions in the liver.

28043

INTERFERON STATUS IN NEWBORNS AFTER INTERFERON TRERAPY OF PREGNANT WOMEN WITH CYTOMEGALOVIRUS AND HERPES SIMPLEX VIRUS INFECTION

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Interferon status were analysed in 52 newborns during the early neonatal period, who were born by mothers infected with cytomegalovirus (CMV) and herpes simplex virus (HSV) and treated with Viferon in the complex therapy against these infections. Comparative group included 44 newborns from mothers with the same infections, who however did not receive Viferon in the complex therapy.

Viferon is a complex preparation, which includes human recombinant interferon-α2b, membrane-stabilising substances and cocoa oil as a base. Viferon was appointed daily from the 1 day after birth, 150 IU twice a day at 12 hours.

In the group of newborns from Viferon-treated mothers with CMV and HSV infections the level of IFN-γ at the first day of life was in two times higher than the same level in the group of newborns from Viferon-not treated mothers (9.5±1.9 IU and 4.4±0.9 IU correspondingly). There was some increasing of IFN-γ at 1.5 times (6.0±0.9 IU) after Viferon therapy in the group of newborns from not treated mothers at the fifth day of life. In the group of Viferon-not treated newborns IFN-γ level was low (3.8±0.9 IU) both in the main group and in the group of comparison.

Thus, obtained data show the necessity of inclusion of Viferon both in complex therapy of HSV and CMV infections of pregnant women and of newborns of the early neonatal period.

28045

Cytokine activity and expression of the soluble tumor necrosis factor receptors in human placenta. Preliminary study on cytokine profile in primary cytomegalovirus infection.

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The placental cells could play a role in natural host resistance either directly or through contributing to local cytokine network at the sites of infection. There is currently a great deal of interest in the role the cytokines play in immunity against human cytomegalovirus (HCMV). In this study we examined the local expression of the soluble tumor necrosis factor receptors: sTNF-RI (p55), sTNF-RII (p75), and cytokine activity (TNF, IFN and IL-1) in organ cultures (OC) of term human chorionic villi and deciduas. Placentas were obtained after normal term delivery or elective cesarean section from uncomplicated pregnancies as well as from women with primary HCMV infection.
Concentrations of IL-1, sTNF-RI and sTNF-RII in OC supernatants were determined by ELISA using commercial kits (R&D Systems). TNF activity was measured by bioassay using L929 cell line, in the presence of the metabolic inhibitor actinomycin D. IFN activity was assayed with a micromethod, consisting in inhibition of the cytopathic effect caused by encephalomyocarditis virus in the human A549 cell line. For the detection of HCMV DNA in decidual and chorionic villous cells, nested-PCR test constructed in the Center of Microbiology and Virology PAS, as well as PCR based TaKaRa Human Cytomegalovirus Detection Set were used.
In OC supernatants, obtained from uncomplicated pregnancies, we found the presence of considerable amounts of biologically active TNF-α. In most of cases sTNF-RI and sTNF-RII are highly expressed by placental tissue cells. It indicates that soluble receptors are shed from decidual and chorionic villous cells *in vitro*. Our study confirms that IL-1 is released constitutively only by decidual cells. In response to HCMV infection placental cells release slightly increased levels of TNF and shed TNF soluble receptors. In contrast, placentas produced very small amounts of IFN as they do not respond to HCMV infection.

28046

VIFERON® - OINTMENT IN TREATMENT OF PAPYLLOMA VIRUS INFECTION

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The broad interest to papylloma virus infection is caused by the high prevalence of benign, precancer and malignant skin neoplasms, associated with it. During last years interferons, which have antiviral activity, were used successfully in treatment of these neoplasms.
We used ointment «Viferon®» (interferon-α2b with antioxidants, vitamins E and C) for treatment of 17 patients with common warts (7), fig warts (5), keratoacanthoma (3) and bovenoid papulosis (2). Patients were from 7 to 72 years old. The ointment was applied to lesions 2-4 times daily. Recovering time varied from 7 to 25 days in case of common warts, 5-28 days in fig warts, 7-30 days in keratoacanthoma. Bovenoid papules were resolved in 35 days in one patient and regressed significantly during 76 days of treatment in another; in the last case they were removed finally with laser. To prevent relapses the treatment of common warts and fig warts with Viferon ointment was continued for 2 weeks after clinical recovery. Tolerance to the ointment was satisfactory in every case. There were no any side effects.

28047

INFLUENCE OF INTERFERON INDUCER AMIXIN AT IL-6 PRODUCTION BY HUMAN PERIPHERAL MONONUCLEAR BLOOD CELLS (hPMBC) IN VITRO.

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IL-6 - is one of the intracellular cooperation proteins secreted by cells at inflamation. It exerts various and essential influence at different organs and systems of organism. The inflammatory cytokines take an active participation in the phatogenesis of different diseases and influence at clinical picture. The aim of our investigation was an experimental study the influence of medicinal preparation - interferon inducer Amixin at the IL-6 production level by hPMBC. HPMBC were resuspended 1:10 in RPMI-1640 medium supplemented with 2% FBS and antibiotics. Cell were incubated with Amixin (50 mkg/ml), LPS (1 mkg/ml), ConA (25 mkg/ml) or (PHA 25 mkg/ml) for 24, 48, 72 h. The level of IL-6 secretion were measured using an ELISA ("Protein Contour", S.-Peterburg). Cytokine status was investigated at 10 donors and 10 patients with the Chronic Fatigue Syndrome caused by Epstein-Barr virus. It was shown that initial spontaneous level of IL-6 at the range of 20,45 to 3897,80 pg/ml was marked in the hPBMC supernatants of all the observed donors and patients, independently of diagnosis. The pick of IL-6 spontaneous production was marked at 72 h. Induction with Amixin suppressed spontaneous IL-6 production (< 20 pg/ml). In control cultures ConA and PHA stimulated its production 2-4 and more times. Since the increase of sick rate and mortality at chronic stress, growing old and many other states correlates with high concentration of IL-6, it is particularly actual the search of remedies, able to suppress IL-6 production and blockade its receptors. In this connection the ability of Amixin to inhibite IL-6 production may be perspective at treatment of deseases connected with it's synthesis.

28048

THE CYTOKINE STATUS IN PATIENT WITH RELAPSING HERPETING INFECTION

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The aim of the study was to investigate the cytokine status on patient with relapsing herpetic infection. Methods: to investigate the interleukins 1β, 4,6,8 and TNF-α by methods immunoferment analysis (IFA) with the native kits (St.Peterburg). To estimate the level of interleukins in the serum of the blood, the cultural habitat of the spontaneous and inducted cells. 21 patient were investigated at the age of 18 to 65 years old: they included 10 women and 11 men. 12 of the patients observed suffered from herpes genitalis. In 3 the manifestation was located on the face. 6 suffered from mixed forms of the disease. Results: the serum level of blood was TNF-α 168.334.3% (N=0-50 pg/ml)IL-1 – 160.6±50.1% (N=<50 pg/ml), IL-4 – 144.3±39.7% (N=<50 pg/ml), IL-6 – 90.4±25.9% (N=<5-50 pg/ml), IL-8 – 70.8±25.6% (N=<50 pg/ml); spontaneous induction of the cytokines was – 198.8±33.9% (N=30-50 pg/ml), 384.6±98.3% (N=<30-50 pg/ml) 41.9±7.4% (N=<20-30 pg/ml), 364.9±146.6% (N=<30-50 pg/ml), 97.9±31.0% (N=30-100 pg/ml) accordingly; and inducted production in the cells habitat was – 194.2±31.0% (N=>500 pg/ml), 385.2±108.3% (N=>1000 pg/ml), 59.8±13.9% (N=>1000 pg/ml), 287.0±100.0% (N=>1000 pg/ml), 167.0±51.5% (N=>1000 pg/ml) accordingly. Conclusions: there was simultaneous rising of the cytokines in the blood serum with the inducted production indexes decreasing in the cells habitat in patient with relapsing herpetic infection. The disbalance of the components of cytokine profile makes impossible adequate immune replay of the elimination of virus-pathogen in relapsing herpetic infection. This results substantiate complex therapy including the antiviral drugs and immunotherapy.

28036

DIFFERENTIAL ROLE OF IL-6 AND IFN-γ ON THE IgG2a RESTRICTION OF POLYCLONAL IMMUNOGLOBULIN AND SPECIFIC ANTIVIRAL ANTIBODIES AFTER INFECTION WITH LACTATE DEHYDROGENASE-ELEVATING VIRUS.

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Lactate dehydrogenase-elevating virus (LDV) triggers, in addition to specific antiviral antibodies, a B lymphocyte polyclonal activation characterized by a marked increase in the production of the IgG2a subclass. Interleukin-6 (IL-6) was secreted shortly after LDV infection most probably by macrophages, but not by T lymphocytes. An increased production of interferon-gamma (IFN-γ) was also detected, with a peak at 18 hours post-infection. The NKT cell population and possibly NK cells were involved in this IFN-γ production. We used IL-6 deficient mice and IFN-γ receptor deficient mice to determine the role of each of the cytokines on the polyclonal and specific antiviral IgG2a production. IL-6 had no influence on the proliferation of B cells. However, IL-6 deficient mice showed a marked decrease in their total IgG2a production when compared to their normal counterparts, whereas their antiviral IgG2a production was not changed. In contrast, IgG2a anti-LDV was reduced in mice lacking IFN-γ receptor, but total IgG2a responses were relatively unaffected by the absence of IFN-γ receptor. These results show that different pathways can lead to the virally-induced IgG2a restriction, depending on the type of antibodies produced. Whether this difference implicates distinct B cell subpopulations or involves different pathways of B cell activation remains to be determined.

28044

INTRON A HAS-FREE SOLUTION FOR TREATMENT OF CHRONIC HEPATITIS C IN INTERFERON-NAÏVE PATIENTS A STUDY OF INTERFERON NEUTRALIZING ANTIBODIES.

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Purpose. To determine the incidence of serum neutralizing antibody (SNA) formation to interferon alfa-2b with a new formulation of Intron A that does not contain human albumin in subjects with chronic hepatitis C (CHC) not previously treated with interferon.

Materials and methods. It was a multinational trial with 53 patients. Eligible subjects were defined as adult male or female with CHC not previously treated with interferon with abnormal ALT levels at least 6 months and at entry. Subjects were to be free of other liver disease, HIV and decompensated cardial, renal or nervous system disease. The patients were treated for 24 weeks with Intron A HAS-free solution 3 MIU TIW subcutaneously and then followed for 4 weeks. Serum for binding antibody was collected prior to treatment and 4 weeks following the end of treatment. Anti-interferon alpha Antibody ELISA from ANAWA Laboratorien was used.

Results. 53 patients were enrolled, 20 of them from Estonia.
7 patients discontinued treatment because of adverse events (none from Estonia) and 3 at their request (1 from Estonia). From Estonian patients 12 were under 30 years old, 18 were male. Viral subtypes were studied in 16 cases type (1b-8 cases). 5 of our patients responded to treatment, 10 relapsed and 4 were nonresponders. Of 42 subjects with post-treatment sample only 1 sample was positive in the bioassay for neutralizing antibody.

Conclusion. The incidence of serum neutralizing antibodies formation to interferon alfa 2b HAS free formulation of Intron A in this study was negligible.

Mode of action of cytokines II

22007

HUMAN MxA PROTEIN ASSOCIATES WITH LaCrosse VIRUS NUCLEOPROTEIN AND PREVENTS ITS ACCUMULATION IN THE GOLGI COMPARTMENT.

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LaCrosse virus (LACV) transcribes and replicates its genome in the cytoplasm of infected cells. Subsequently, viral ribonucleoprotein complexes (vRNPs) are formed that gain their envelope by budding through membranes of the Golgi apparatus. Cytoplasmic MxA protein inhibits LACV multiplication at a still ill-defined replication step, as demonstrated in MxA-expressing Vero cells (1). We now report that MxA binds to the nucleoprotein of LACV and prevents its accumulation in the Golgi compartment. Thus, MxA colocalizes with LACV nucleoproteins in large intracytoplasmic deposits, whereas the viral glycoproteins are transported normally into the Golgi compartment. In infected control cells lacking MxA, the viral nucleoprotein translocates also into the Golgi, as expected. To show the specificity of this effect, we used MxA(E645R), a mutant of MxA with altered antiviral specificity. MxA(E645R) inhibits influenza virus replication but not LACV. MxA(E645R) did not interfere with the translocation of LACV nucleoprotein into the Golgi compartment. Moreover, coimmunoprecipitation experiments using a nucleoprotein-specific antibody demonstrated that the nucleoprotein was precipitated together with wild-type MxA but not with MxA(E645R). Our data demonstrate a direct interaction of MxA with LACV nucleoproteins leading to missorting of this vRNP component. It is conceivable that MxA interferes with the translocation of newly formed vRNPs into the Golgi compartment and thereby prevents the maturation of progeny virus particles.

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22005

Immune Response in Stat2 Knockout Mice

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Stat2, a critical component of the type I IFN transcription factor ISGF-3, is also an unusual member of the STAT family. It is the only STAT that fails to homodimerize or bind members of the GAS family of enhancers. To explore the unique properties of Stat2 and the role it plays in type I IFN signaling, it was targeted for deletion by homologous recombination. Stat2 null mice and their tissues are highly susceptible to viral infection and unresponsive to type I IFNs. These mice exhibit a number of additional defects that highlight a more pervasive role for Stat2 and type I IFNs in regulating the immune response. This includes evidence for a type I IFN autocrine/paracrine loop that is important for antiviral activity, target gene expression and lymphocyte proliferation. In addition, Stat2 null tissues are more susceptible to viral infection than Stat1 null tissues, suggesting that Stat2 may transduce some signals that are independent of the Stat1:Stat2 heterodimer found in ISGF-3. Unexpectedly, the IFN-α dependent upregulation of MHC I is Stat2 dependent in MEFs but Stat2 independent in macrophages. This suggests that type I IFNs are able to transmit important tissue specific signals through both Stat2 dependent and independent pathways. In sum these observations demonstrate that Stat2 plays a considerably more pervasive role in the regulation of the immune response than had been previously recognized.

22013

INTERFERON-INDUCIBLE DOUBLE-STRANDED RNA-SPECIFIC ADENOSINE DEAMINASE (ADAR1): NOVEL REGULATION BY INTERFERON AND EDITING OF GLUTAMATE AND SEROTONIN RECEPTOR PRE-mRNAs

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ADAR1 is a double-stranded RNA-specific adenosine deaminase that catalyzes the C-6 deamination of adenosine to generate inosine in viral and cellular RNAs. We have isolated and characterized cDNA and genomic clones of ADAR1. The ~7-kb cDNA predicts a protein with three copies of the double-stranded RNA-binding motif (dsRBM) and two copies of a Z-DNA binding domain in addition to the deaminase catalytic domain. Mutational analyses confirmed the identity of the catalytic domain and the dsRNA and Z-DNA binding domains. The human *ADAR1* gene was mapped to chromosome 1q21 and the mouse gene to 3F2. The *ADAR1* gene spans ~40-kb and includes 16 exons. Two forms of the ADAR editing enzyme were identified: an interferon (IFN) inducible ~150-kDa protein present in both the cytoplasm and nucleus and a constitutively expressed N-terminally truncated ~110-kDa protein found predominantly in the nucleus. Cloning and sequence analyses of 5'-RACE cDNA clones from human tissue and cultured cells established a linkage between exon 2 of *ADAR1* and alternative exon 1 structures. Different promoters, one IFN-inducible and the others constitutively active, were identified from which the alternative exon 1 structures initiate. Expression of alternative ADAR1 transcripts in untreated and IFN-treated cells and tissues was assessed by nuclease protection, RT-PCR and Northern analyses. ADAR1 transcripts displayed tissue selectivity in their expression. Transient transfection analyses led to the identification of two classes of functional ADAR1 promoters, a constitutively active P_c promoter and an IFN inducible ISRE-containing P_I promoter. The alternative exon1-containing transcripts possessed the coding capacity for the inducible ~150-kDa ADAR and the constitutive ~110-kDa ADAR proteins. The IFN-inducible ADAR deaminase efficiently edited *in vitro* the pre-mRNAs for two neurotransmitter receptors, the R/G site of the GluR-B glutamate receptor and the A-site of the 5-HT2cR serotonin receptor. These sites, which affect function of the receptors, also were selectively edited in rat brain. Finally, mutational analysis revealed a different relative importance of the three dsRBM motifs for editing GluR-B and 5-HT2cR cellular substrates as well as dsRNA substrates. *(Supported by NIAID, NIH).*

22006

CCR5: A SIGNALING SCAFFOLD REGULATED BY RANTES, MYXOMA VIRUS AND RGS-6.

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Chemokine receptor engagement activates signaling cascades implicated in lymphocyte development and homeostasis, wound healing, hemopoiesis, and, depending on the circumstances, either the clearance or transmission of infectious organisms. In this report we provide direct evidence for the RANTES-CCR5 dependent recruitment and activation of distinct protein kinases in T cells: Jak2 and Jak3; the Src kinase, $p56^{lck}$; the MAP kinases, p38 and MAPKAP kinase 2. Whereas activation of Jak2, Jak3 and $p56^{lck}$ requires their phosphorylation on tyrosines, activation of p38 requires both threonine and tyrosine phosphorylations. The hierarchical p38 signaling pathway invokes serine phosphorylation of target substrates. Clearly, the RANTES-dependent sequestering of different signaling molecules to CCR5 provides for signal integration or reciprocal modulation of interacting signaling pathways. Our data suggest that RANTES-dependent recruitment of $p56^{lck}$ and Jaks to CCR5 may result in complex patterns of interactions among signaling molecules, that may allow for cross-talk between G protein-coupled signaling cascades and non-G protein linked cascades. Additionally, we provide evidence that activation of CCR5, in the context of receptor tyrosine phosphorylation, is a pre-requisite for productive poxvirus replication: Myxoma virus induces the rapid phosphorylation of CCR5 on tyrosine residues, the association of CCR5 with phosphorylated Jaks and $p56^{lck}$ and subsequent Stat and IRS activation. In contrast to CCR5 activation effected by HIV Env protein, these myxoma virus-inducible phosphorylation events are not sensitive to Ptx treatment. In CCR5-expressing cells that are non-permissive for myxoma virus infection, myxoma virus fails to activate CCR5 and invoke this tyrosine phosphorylation cascade. We infer that viral infectivity is dependent on non-G-protein coupled CCR5-mediated signal transduction pathways triggered by the infecting myxoma virus particle. This provides a novel post-binding mechanism by which viruses can co-opt a cellular receptor to permit productive virus infection. Finally, we provide compelling evidence for the regulation of CCR5 activity: (1) mediated by RGS-6, that limits sensitivity to RANTES-inducible T cell proliferation and (2) mediated by $p56^{lck}$, that regulates myxoma virus replication.

22011

COMPROMISED ADAPTIVE AND INNATE IMMUNE RESPONSES IN TYK2-DEFICIENT MICE

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Tyk2 was the first described member of the Janus protein tyrosine kinase (Jaks) family[1,2].
Studies in different cell lines have shown that Tyk2 is activated in response to a variety of cytokines, including IFN α/β, IL10, IL12, TPO, G-CSF and the gp130 receptor subunit utilizing cytokines [3].
To assess the role of Tyk2 in vivo, we have generated Tyk2 deficient mice. $Tyk2^{-/-}$ mice do not show gross morphological abnormalities, are fertil and are not to be affected by low enviromental microbial load. In contrast to other Jaks, where inactivation leads to a complete loss of the respective cytokine receptor signal, $Tyk2^{-/-}$ mice display reduced responses to IFNα/β and IL-12 and selective deficiency in Stat3 activation in these pathways. Although no association of Tyk2 with the IFNγ receptor has been reported, IFNγ signaling is also impaired in $Tyk2^{-/-}$ mice. Infection studies with high doses of VSV, Vaccinia virus and LCMV show that $Tyk2^{-/-}$ mice are unable to clear vaccinia virus and show a reduced T cell response after LCMV challenge. $Tyk2^{-/-}$ macrophages fail to produce nitric oxide upon lipopolysaccharide induction. These data imply a selective contribution of Tyk2 to the signals triggered by various biological stimuli and cytokine receptors. Furthermore the data presented strongly suggest a function of Tyk2 as modulator, amplificator or selector rather than a role as a primary activator in the different cytokine signaling pathways.
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22001

Induction of human endogenous IFNA genes requires IRF-7 and IRF-3.

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IRF-7 plays an essential role in virus-activated transcription of IFNA genes. Since multiple IRF-Es are present in the IFNA promoter region, the involvement of additional IRFs in the transcriptional activation of IFNA genes was investigated. By generating an IRF-7 dominant negative (DN) mutant which does not contain either DNA-binding domain or transactivation domain, we found that overexpression of IRF-7DN efficiently suppressed the expression of both endogenous IFNA and IFNB genes. The mechanism underlying the IRF-7DN-mediated transcriptional suppression revealed that IRF-3 is one of the targets for IRF-7DN. IRF-7DN was found to interact with IRF-3 in vivo in virus infected cells. In the presence of IRF-7DN, the binding of both IRF-3 and IRF-7 to the IFNA1 VRE was markedly reduced. These results suggest that IRF-3 also participate in the transcriptional activation of IFNA genes possibly by forming a heterodimer with IRF-7. In consistent with this conclusion, we found that an introduction of 5 nucleotides in between two of the IRF-Es in VRE significantly decrease the inducibility of this mutant IFNA1 reporter gene. Furthermore, the expression of endogenous IFNA genes could also be inhibited in the presence of IRF-3 ribozyme which specifically disrupts the IRF-3 mRNA. Interestingly, in P2.1 cells which express low level of IRF-3, we found that overexpression of IRF-3 and IRF-7 failed to restore the IFNA gene expression. An additional IRF required for the IFNA gene expression in these cells will be discussed.

08001

Extinction of melanogenesis and expression of glial cell markers in F10.9 melanoma treated with IL6RIL6 .

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The murine B16/F10.9 melanoma cells proliferate rapidly and produce large amounts of eumelanin in vitro. Stimulation of the gp130 transmembrane signaling molecule with a fusion protein of interleukin-6 receptor and interleukin-6 (IL6RIL6) leads to 80% of growth reduction in 72 hours and to extinction of melanogenesis. Concomitantly, cells acquire a morphology resembling the one of glial cells. We observed that the intermediate filament protein GFAP, whose normal site of expression is astrocytes or glial cell precursors, is already induced in protein extracts by 12 hours of treatment with IL6RIL6. The mRNA and protein levels peak at 24 hours and mRNA decreases at 48 hours. No expression of GFAP protein is seen in untreated extracts. Between 24 and 48 hours of treatment, the levels of myelin basic protein mRNAs increase strongly. The CNPase protein, abundant in myelin, is often expressed in melanoma cells and can be detected in non-treated F10.9 cells. However, CNPase levels are strongly induced by 48-72 hours of treatment with IL6RIL6. We found that extinction of melanogenesis is mediated by a strong and stable reduction in mRNAs levels of the HLH- leucine zipper transcription factor MITF, and that the MITF promoter activity is down regulated. The paired homeodomain factor, Pax3, which transactivates the MITF promoter, is also down regulated at the protein and mRNA levels. Common precursors to melanocytes and glial cells in neural crest express Pax3, which persists in melanocytes, but was shown to decrease in glial precursors acquiring myelin gene expression. The F10.9 cell model supports a role of gp130 receptor signaling in neural crest cells differentiation and in expression of myelin genes, through regulation of Pax3.

Cytokines and interferons in autoimmunity

30004

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Abstract not available at time of printing.

30003

EFFECTS OF PEG sTNF-RI, IL-1ra, OR THE COMBINATION IN TNF-α KNOCKOUT MICE EXPRESSING A MUTANT TRANSGENIC FORM OF MURINE TRANSMEMBRANE TNF-α.

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TNF-α knockout mice expressing a mutant transgenic form of murine TNF-α (TgA86) develop severe arthritis characterized by neutrophil infiltration, bone and cartilage destruction. TgA86 mice (9-12/group) were treated for 4 weeks (q2d) with the agents listed below:

Treatment	Mean±Total Histologic Score (6 joints scored 0-5)	% inhibition from control *p<0.05, T test to control
Vehicle ip/	65.1±1.62	0
PEG sTNF-RI	20.0±1.13	69*
Enbrel	24.4±5.2	63*
Vehicle ip/sc	60.2±3.0	0
PEG sTNF-RI	25.2±4.8	58*
IL-1ra	59.3±2.2	1
PEG sTNF-RI+IL-1ra	15.9±3.9	74*
Vehicle po	64.7±4.1	0
Dexamethasone	27.4±5.2	58*

These results demonstrate effective inhibition of arthritis associated with over-expression of membrane bound TNF (in the absence of soluble TNF) by both sTNF-RI, sTNF-RII (Enbrel) and dexamethasone but not IL-1ra. Dose responsive effects with these agents and plasma levels of drug associated with efficacy including DEX will be discussed. Combination treatment resulted in an enhanced benefit over that seen with PEG sTNF-RI alone thus suggesting involvement/induction of IL-1β in this disease process.

30006

INFLAMMATORY DISEASES IN IL-1 RECEPTOR ANTAGONIST-DEFICIENT MICE

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We have previously reported that IL-1ra-deficient mice, under specific pathogen-free conditions, develop gross transmural inflammation at stress sites of the high pressure vasculature. We are developing our own susceptible inbred strain, Sf3 from our previously outbred colony. We now show evidence of highly localised IL-1 driven activation of E-selectin expression in the aortic endothelium and vasa vasorum. There is heavy accumulation of F4/80 positive cells (macrophages) and CD4+ T cells, and few CD8+ T cells are also present. Intracellular accumulation of IFNγ is observed, but no IL-4 or IL-5 has yet been detected, implying a strongly Th1 driven pathology. In confirmation of the work of others, we find that our own null allele of IL-1ra also gives a high incidence of a rheumatoid arthritis-like pathology when crossed onto Balb/c, but not onto C57BL/6. We have also not observed signs of arthritis in over 400 homozygous animals of our own outbred colony. The lack of any defined, localised pathology in C57BL/6, the general reproducibility of joint pathology in Balb/c (regardless of the source of the null-allele and the site of rearing) and the presence of a distinct inflammatory pathology in Sf3, give clear evidence of the importance of genetic background in the development of localised inflammatory diseases in mice that lack IL-1ra; an antinflammatory component that has always been presumed to have a general function in resolving inflammation. In our own colony, IL-1ra deficient mice that were culled at 600 days, like the youngest animals, were found to have active lesions or aortic scarring. In tracking age of morbidity (varying from 50 - 600 days) as a result of arterial inflamation in our own colony, we find strong heritability and a good fit to the presence of one or two genetic modifiers. We have found, though, that age of disease onset does not appear to segregate with *H-2* haplotype.

30001

THERE IS A GENETIC PREDISPOSITION TO LOW IL-10 PRODUCTION IN CHILDREN WITH EXTENDED OLIGOARTICULAR JUVENILE IDIOPATHIC ARTHRITIS

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We have shown that the IL-10 5' flanking region ATA haplotype is associated with low IL-10 production and that children with the ATA haplotype are twice as likely to develop extended oligoarticular JIA compared to oligoarticular JIA. To investigate this association further, we used the transmission disequilibrium test (TDT) in 105 families. There was a significant increase in transmission of the ATA haplotype to patients with oligoarticular onset JIA (p = 0.05). There was also a significant increase in the transmission of both the ACC and the ATA haplotype to patients with uveitis (p=0.014 each). IL-10 production cannot be measured directly in JIA patients because of the treatment they receive. To investigate whether children with extended oligoarticular JIA produce less IL-10 during inflammation than children with oligoarticular JIA, parental IL-10 production was used as a marker for childhood IL-10 production. This is valid as 84% of the variability in IL-10 production is genetically determined. IL-10 production in the parents was measured using LPS stimulated whole blood culture. We showed that mean IL-10 production was lower in the parents of those with extended oligoarticular JIA (N=14, Mean = 2109.2, SE 251) than those with oligoarticular JIA (N=12, mean = 3161.7, SE=411) and the results are statistically significant (p=0.034, CI 88 to 2016). This suggests that stimulated IL-10 production is lower in children with extended oligoarticular JIA. In addition, there was an increase in the frequency of ATA containing genotypes in the parents of children with extended oligoarticular JIA compared to controls (p<0.02) but not in those with oligoarticular JIA. These findings strongly suggest that children with arthritis can have a genetic predisposition to low IL-10 production and a concomitant increase in their risk of developing either extended oligoarticular JIA or uveitis.

30002

ENHANCED SUPPRESSION OF CYTOKINE SIGNALING IN INFLAMED SYNOVIA FROM IL-6 DEFICIENT MICE RESISTANT TO DEVELOPING CHRONIC ARTHRITIS.

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Recently we showed that IL-6 played an important role in the maintenance of joint inflammation in two experimental models of rheumatoid arthritis. Both the irritant induced zymosan - and the immunologically mediated antigen-induced gonarthritis developed into a severe and chronic joint inflammation in wildtype (WT) mice lasting 3-4 weeks. However, in IL-6 gene knockout (KO) mice the acute joint inflammation did not turn into a chronic inflammatory joint disease. In order to elucidate the mechanism behind the enhanced remission in IL-6 KO mice, the expression of suppressors of cytokine signaling (SOCS) and activation of signal transducer and activator of transcription (STAT)3 was evaluated during the course of zymosan-induced arthritis (ZIA). In WT mice, mRNA expression of SOCS1 and SOCS3 was increased at day 2, 7, 14, 21, and 28 of arthritis. As expected the expression of mRNA of SOCS1 was further increased, 6 fold in IL-6 KO mice at day 14 of ZIA. Next we studied the effect of cytokine signaling suppression by analyzing STAT3 phosphorylation in synovial tissue using westernblotting. During the initial phase of inflammation (day 1 and 2) STAT3 became phosphorylated in both WT and IL-6 KO mice. In this phase of ZIA, the inflammatory response did not differ between both mouse strains. However, during the chronic phase of arthritis (day 7, 14), STAT3 phosphorylation was evident in WT mice but markedly diminished in IL-6 KO mice. No differences were found between WT and KO mice in the total amount of STAT3.
This study showed for the first time the expression of SOCS1 and SOCS3 in inflamed synovial tissue from arthritic mice. Furthermore, we found that IL-6 played an important role in the maintenance of a chronic arthritic process probably via continued STAT3 phosphorylation as a consequence of reduced SOCS1 mediated suppression.

30005

DNA VACCINATION AGAINST IL-18 DECREASES AUTOIMMUNE ALTERATIONS AND PROLONGS SURVIVAL IN MURINE LUPUS

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The lupus-like autoimmune syndrome of MRL *lpr/lpr* mice is characterized by progressive lymphadenopathy and autoantibody production and leads to early death due to renal failure. Among Th1-dependent cytokines, IFNγ apparently plays a pivotal role in the abnormal cell activation and in the fatal development of the *lpr/lpr* autoimmune disease. Here, the involvement of the IFNγ-inducing factor IL-18 has been examined during the progression of the *lpr/lpr* syndrome. At variance with normal cells, IL-18 is constitutively produced by *lpr/lpr* lymphocytes. In addition, *lpr/lpr* cells are hyperreactive to IL-18 both in terms of IFNγ production and proliferation. Thus, deregulated IL-18 production and responsiveness could be among the causes of pathogenic IFNγ production.
The contribution of IL-18 to the pathogenic processes of the autoimmune syndrome has been assessed in a system of *in vivo* inhibition of IL-18 in the *lpr/lpr* mouse. Young *lpr/lpr* mice were vaccinated against autologous IL-18 by repeated administration of a cDNA coding for murine IL-18. Vaccinated mice, which produced autoantibodies to murine IL-18, showed a significant decrease of several autoimmune parameters (excessive IFNγ production, spontaneous lymphoproliferation, NK cell activation) and of the endorgan disease (glomerulonephritys and renal damage). Eventually, early mortality was significantly delayed and decreased in anti-IL-18 vaccinated mice.
It is concluded that IL-18 plays a major pathogenic role in the Th1-dependent autoimmune syndrome of *lpr/lpr* mice and that anti-IL-18 approaches could be beneficial in the development of effective therapeutic strategies to autoimmune diseases.

30007

NEUTRALIZATION OF IL-18 EXERTS ANTI-INFLAMMATORY ACTIVITY IN EXPERIMENTAL COLITIS IN MICE

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IL-18 initially decribed as IFNγ-inducing factor is expressed in the inflamed mucosa of patients with inflammatory bowel disease. In the present study we investigate the anti-inflammatory potency of neutralizing anti-IL-18 antiserum in experimental colitis in mice. For induction of colitis Balb/c mice received 3.5% dextran sulfate sodium (DSS) for 10 days as drinking supply. Anti-IL-18 antiserum was injected on days 1 and 5 or on days 1, 4 and 8 intraperitoneally. The severity of the clinical score was determined by weight loss, stool consistency and bleeding (range from 0 = healthy to 4 = maximal activity of colitis). Anti-IL-18 treatment resulted in a dose-dependent reduction of the severity of the colitis (3.4 ± 0.2 in the untreated DSS-fed mice compared to 1.7 ± 0.2 in the anti-IL-18 antiserum-treated DSS-fed mice, n = 8, p = 0.003) and histologic scores. Colon shortening, which develops during DSS-induced colitis, was partially prevented in the anti-IL-18-treated groups. In the eluate of colon tissue homogenates, IFNγ concentrations were lower in the anti-IL-18-treated DSS-fed mice (28 ± 8 pg IFNγ/ng protein) compared to untreated DSS-fed mice (48 ± 10 pg IFNγ/ng protein; n = 8, p = 0.011). This suppressive effect of anti-IL-18 antiserum on IFNγ synthesis was also observed *ex vivo* in the supernatant of either unstimulated (24 ± 5 pg IFNγ/µg protein in the anti-IL-18-treated DSS group compared to 16 ± 5 pg IFNγ/µg protein in the untreated DSS group; n = 8, p = 0.029) or IL-12-stimulated (81 ± 14 pg IFNγ/µg protein in the anti-IL-18-treated DSS group compared to 65 ± 23 pg IFNγ/µg protein in the untreated DSS group; n = 8, p = 0.048) colon organ culture. From these results, we conclude that IL-18 is a pivotal inflammatory mediator in colitis. Suppression of IL-18 represents a rational strategy for anti-inflammatory therapy in inflammatory bowel disease.

30014

DOUBLE-STRANDED RNA INCREASES CYTOKINE-INDUCED APOPTOSIS IN PANCREATIC β-CELLS

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Type 1 diabetes mellitus (T1DM) is an autoimmune disease caused by progressive destruction of insulin-producing pancreatic β-cells. Both viral infections and cytokines (specially IL-1β and INFγ) have been suggested as potential mediators of β-cell apoptosis in early T1DM. We presently investigated whether the viral replicative intermediate double stranded RNA (dsRNA; synthetic poly IC), modifies the effects of IL-1β and INFγ on gene expression and viability of FACS-purified primary rat pancreatic β-cells. For this purpose, β-cells were exposed for 6 h (gene expression) or 6-9 days (viability) to dsRNA and/or IL-1β and INFγ. Gene expression was evaluated by RT-PCR and apoptosis/necrosis by nuclear dyes. DsRNA increased the expression of Fas and Mn superoxide dismutase mRNAs by nearly 10-fold (n = 3). This effect was additive to IL-1β-induced gene expression, but was not modified by INFγ. IL-1β, but not dsRNA or IFNγ alone, induced expression of iNOS and consequent NO production. A combination of dsRNA + IFNγ, however, lead to iNOS expression and NO formation. DsRNA + IFNγ-induced iNOS expression requires NF-κB activation, as suggested by transfection experiments with iNOS promoter-luciferase reporter constructs into β-cells (n = 3; >70% decrease in iNOS promoter activity following NF-κB binding site inactivation). Neither IL-1β, dsRNA or IFNγ alone induced β-cell death after a 6-9 days exposure. On the other hand, combinations of IL-1β + IFNγ, dsRNA + IFNγ or IL-1β + dsRNA induced a significant increase in the number of apoptotic β-cells (2-3 fold increase in the apoptosis index; P<0.05-0.01 vs controls; n = 5). The iNOS inhibitor NMA significantly decreased IL-1β + dsRNA, but not dsRNA + IFNγ, induced apoptosis. In conclusion, dsRNA, alone or in combination with cytokines, modifies the expression of several genes in pancreatic β-cells. Two of these genes, Fas and iNOS, may contribute to β-cell death. The transcription factor NF-κB is required for dsRNA-induced iNOS expression. DsRNA has also an additive effect on cytokine-induced β-cell apoptosis. These findings suggest that viral intermediates, in synergism with local cytokine production, may play an important role for β-cell apoptosis in early T1DM.

30009

EXPRESSION OF THE CHEMOKINE MONOCYTE CHEMO-ATTRACTANT PROTEIN-1 IN CULTURED ISLET CELLS AND IN PRE-DIABETIC NOD MICE ISLETS

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The chemokine monocyte chemoattractant protein-1 (MCP-1) may participate in the recruitment of mononuclear cells into the pancreatic islets in early type 1 diabetes. We analyzed the signal transduction for IL-1β-induced MCP-1 expression in rat β-cells, the MCP-1 mRNA expression and protein release by human islets, and the MCP-1 mRNA expression in pre-diabetic NOD mice. FACS-purified rat β-cells were cultured for 6 h with IL-1β and/or the MAPK inhibitors SB203580 (p38i) or PD098059 (MEKi). Human islets from 5 different donors were cultured for 6-72 h with the cytokines IL-1β (50 U/ml) and/or IFN-γ (1000U/ml). MCP-1 mRNA content was determined by RT-PCR and protein release by ELISA. MCP-1 mRNA expression in islets from male and female NOD mice (2-12 weeks of age), or from non-diabetes prone BALB/c mice, was measured by real time RT-PCR. IL-1β induced MCP-1 mRNA expression in rat purified β-cells. p38i or MEKi alone partially inhibited (20-40%) this induction, while together they decreased by 70% MCP-1 expression (n=4, $p<0.01$ vs. IL-1β). IL-1β induced both MCP-1 mRNA expression and a 3-fold increase in medium MCP-1 protein accumulation in human islet cells ($n = 5$; $p<0.05$ vs. control). This effect was neither stimulated by IFN-γ nor prevented by iNOS blockers. In vivo there was an age-related increase in MCP-1 mRNA expression in islets from male and female NOD mice, reaching a peak at 8 weeks (15-fold increase vs. BALB/c mice; $p<0.05$). In conclusions, MCP-1 is induced by IL-1β in rat and human islet cells. Both ERK1/2 and p38MAPK, but not NO, are involved in IL-1β-induced MCP-1 expression. In NOD mice MCP-1 mRNA expression increases with age, peaking at the early phases of insulitis. These data suggest that chemokines, produced by the target cells of an autoimmune attack, may contribute for the local accumulation of immune competent cells.

30008

DISEASE INDUCIBLE IL-1RA OVEREXPRESSION RESULTS IN A MORE EFFICIENT INHIBITION OF THE COLLAGEN INDUCED ARTHRITIS MODEL THAN CONSTITUTIVE OVEREXPRESSION OF IL-1RA

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The constitutive cytomegalovirus (CMV) promoter is frequently used in adenoviral vectors (Ad) to overexpress cytokines/cytokine inhibitors. The major disadvantages of the CMV promoter are, the uncontrollable activity and the rapid silencing by methylation of the CpG islands. Cellular promoters are less vulnerable to silencing, and a promoter can be chosen that responds to cellular signals induced by the disease, permitting autoregulation of drug dose by natural homeostatic mechanisms. The polyarticular Collagen Induced Arthritis (CIA) model was used to compare a disease inducible promoter to the constitutive CMV promoter. We used an adenoviral vector (Ad.), containing the two component-inducible system in which the complement factor 3 (C3) promoter, which is upregulated by inflammatory mediators, regulates production of HIV transactivator of transcription (Tat), and the Tat protein then stimulates expression of the human IL-1ra gene. The HIV LTR promoter is also vulnerable for CpG island methylation and silencing, but the Tat protein could counteract this mechanism. DBA/1 mice were immunized with bovine type II collagen on day 0 and boostered on day 22. On day 22, mice without any signs of arthritis were selected and bilaterally injected into the knee joints with 10^7 pfu Ad.CMV-Luc, Ad.CMV-IL-1ra, or Ad.C3-Tat/HIV-IL-1ra. The injected knee joints and ipsilateral paws were scored macroscopically for signs of arthritis, on day 14. The C3-Tat/Hiv promoter driven IL-1ra overexpression resulted in significantly better inhibition of CIA then did CMV-driven overexpression. Furthermore, the overexpression of IL-1ra in the knee joints also resulted in an inhibition of the CIA in the ipsilateral paws. Our data demonstrate the feasibility of the two component transactivator system to overexpress IL-1ra for treatment of arthritis, and show that this system is significantly better than the CMV promoter.

Treatment	Paws			Knees		
	Incidence	Arthritis score	P value	Incidence	Arthritis Score	P value
A	22/22	1.7±0.08	-	22/22	1.35±0.1	-
B	18/22	0.94±0.16	P<0.001	15/22	1.07±0.19	ns
C	10/24	0.45±0.16	P<0.001 / P<0.016*	4/24	0.25±0 12	P<0.001/ p<0.002*

A) Ad.CMV-luc, B) Ad.CMV-IL-1ra, C) Ad.C3-tat/Hiv-IL-1ra.
*To the Ad.CMV-IL-1ra group.

30017

SYSTEMIC IL-18 LEVELS AS AN ACCURATE INDEX OF SEVERITY FOR CROHN'S DISEASE

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IL-18 is a Th1 polarizing cytokine that has been implicated in several chronic inflammatory diseases, including Crohn's Disease (CD). Within the gut mucosa, IL-18 is expressed by intestinal epithelial cells and lamina propria mononuclear cells (LMPC). IL-18 is synthesized as a 24 kD biologically inactive precursor that is subsequently cleaved into a 18 kD mature, bioactive molecule. In the present study, we investigated if circulating and/or locally produced total IL-18 levels and production of pro- and/or mature forms correlate with disease severity in CD and ulcerative colitis (UC), the two major forms of inflammatory bowel disease. Assays for IL-18 protein were performed on plasma (N=8/grp) and mucosal biopsies (N=13/grp) from patients with active and inactive disease as well as involved and non-involved areas of inflammation, respectively. Our results show that plasma IL-18 levels correlate with disease activity in CD (act-34.3±9.9 vs inact-14.4±5.7 pg/ml), but not UC (act-12.4±4.2 vs inact-8.3±3.7 pg/ml), compared to control (C) patients (14.0±4.6 pg/ml). Total IL-18 from mucosal biopsies correlate to disease severity in CD (inv-99.1±12.7 vs noninv-70.6±15.0 ng/ml) but also in UC (inv-245.8±25.3 vs noninv-98.4±10.6 ng/ml), compared to C (133.2±7.9 ng/ml); however, CD levels are reduced compared to UC or C at the local vs systemic level. Higher proportions of bioactive to total IL-18 detected by Western blot in CD mucosal biopsies may provide an explanation for this discrepancy due to active export of the mature form: CD (27.55±2.55) vs UC (14.4±2.05), or C (12.6±2.4). In addition mRNA levels of IL-18 binding protein were detected at higher levels in CD LMPC (87.8±17.3) compared to UC (60.7±13.4) or C (69.8±24.5) and IL-18 binding protein may decrease detected IL-18 levels. These data demonstrate that systemic IL-18 levels, but not gut mucosal levels may be an adequate index of CD disease severity.

30018

MUCOSAL CYTOKINE PROFILES IN THE TNF ΔARE MODEL OF CROHN'S DISEASE (CD)-LIKE ILEITIS

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Mice carrying a deletion in the 3'AUR region of the TNF gene (TNF ΔARE) develop transmural ileal inflammation closely resembling human CD, a prototypic Th1-mediated disorder. The aim of this study was to determine mucosal cytokine profiles and the response to conventional CD therapies in TNF ΔARE mice. Ileal tissues from mutant and WT littermates were harvested and processed for immunohistochemical, protein and mRNA analyses of the Th1 polarizing cytokines TNF, IFNγ, IL-12 and IL-18. TNF ΔARE mice were also treated with dexamethasone (DexM, 100 mg/day X 3d, i.p.) or vehicle; ileal and serum samples were obtained for histology and sICAM-1 measurements (marker for gut inflammation), respectively. Intense staining for TNF and IL-18 was observed in the gut mucosa of TNF ΔARE mutant mice and correlated with disease severity. Similarly, ileal TNF and bioactive IL-18 (by Western blot) were elevated in mutant vs. WT mice. Both IFNγ and IL-12 were detected by immunohistochemistry; however, low to undetectable protein levels (2.5(SLD)-55 pg/ml) were measured in mutant mice. Interestingly, while steady-state levels of TNF and IL-12 transcripts increased, IL-18 mRNA levels appeared to diminish, with increased ileal inflammation Treatment of mutant mice with DexM significantly decreased ileal inflammation (inflammatory index = 4.5±1.3 vs. 15.2±1.2, $p<0.02$) as well as serum sICAM-1 levels (from 59.0±12.4 to 36.0±6.5 ng/ml, $p<0.02$ vs. from 58.0±1.6 to 57.0±7.2 ng/ml, n.s.). These results demonstrate striking similarities between the TNF ΔARE model of ileitis and human CD, making this model a powerful tool for investigating pathogenic mechanisms of TNF-induced CD.

30011

Development of Chronic Inflammatory Arthropathy Resembling Rheumatoid Arthritis in IL-1 Receptor Antagonist Deficient Mice

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Interleukin-1 (IL-1) is a proinflammatory cytokine that plays important roles in inflammation, host defense, and the neuro-immuno-endocrine network. IL-1 receptor antagonist (IL-1ra) is an endogenous inhibitor of IL-1 and is supposed to regulate IL-1 activity. However, its pathophysiological roles in a body remain largely unknown. To elucidate the roles of IL-1ra, IL-1ra deficient mice were produced by gene targeting, and the pathology was analyzed on different genetic backgrounds. We found that all of the mice on a BALB/cA background, but not on a C57BL/6J background, spontaneously developed chronic inflammatory polyarthropathy. Histopathology showed marked synovial and periarticular inflammation, with articular erosion caused by invasion of granulation tissues closely resembling that of rheumatoid arthritis in humans. Moreover, elevated levels of antibodies against immunoglobulins, type II collagen, and double-stranded DNA were detected in these mice, suggesting development of autoimmunity. Proinflammatory cytokines such as IL-1β, IL-6 and TNF-α were overexpressed in the joints, indicating regulatory roles of IL-1ra in the cytokine network. We thus show that IL-1ra gene deficiency causes autoimmunity and joint-specific inflammation and suggest that IL-1ra is important in maintaining homeostasis of the immune system. Roles of IL-1 and IL-1ra in pathogenesis of the disease will be discussed.

30012

AN IFN-γ INDEPENDENT PRO-INFLAMMATORY ROLE OF IL-18 IN MURINE ARTHRITIS

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Interleukin-18 (IL-18) is a member of the IL-1 family of proteins that exerts pro-inflammatory effects. IL-18 is a pivotal cytokine for the development of Th1 responses and induces production of pro-inflammatory cytokines such as TNFα and IL-1 in vitro. The goal of the present study was to investigate the role of endogenous IL-18 in murine streptococcal cell wall (SCW) induced arthritis. C57Bl/6, Balb/C and IFN-γ deficient mice were injected with 2mg rabbit anti-murine IL-18 antibodies, shortly before induction of arthritis by intraarticular injection of 25μg SCW fragments into the right knee joint. Joint swelling and chondrocyte synthetic function was analyzed several days after induction of arthritis. Suppression of joint swelling was noted at days 1 and 2 of SCW arthritis after blockade of endogenous IL-18. Severe inhibition of chondrocyte proteoglycan (PG) was seen in the vehicle treated control animals, whereas markedly reduced inhibition of chondrocyte PG synthesis was found in the anti-IL-18 exposed animals. Analysis of local cytokine concentrations after neutralization of IL-18 during onset of SCW arthritis showed that TNFα, IL-18 and IL-1β was decreased and IL-1Ra was enhanced. Induction of SCW arthritis in IFN-γ deficient mice showed that IFN-γ was not involved in the onset of SCW arthritis and concomitant inhibition of chondrocyte PG synthesis. Blockade of endogenous IL-18 in IFN-γ deficient mice showed similar results as found in wild type animals indicating a role for IL-18 that is IFN-γ independent. The present study indicates that IL-18 is a pro-inflammatory cytokine during the onset of murine SCW arthritis. This inflammation-promoting role of IL-18 is IFN-γ independent.

30016

NEW PREPARATION IN RHEUMATOID ARTHRITIS TREATMENT

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Rheumatoid arthritis (RA) - chronic remittent autoimmune disease with excessive production proinflammatory cytokines (IL-1, TNF-α, IL-6, IL-8, GM-CSF). Previosly we have show that in patients with RA was detected IFN-system deficiency which expressed depression of IFN-α and IFN-γ producing capacity of leukocytes in vitro. Early in the treatment of RA we used the IFN-α2b preparations (Reaferon, Realdiron), IFN-γ (Gammaferon) and IFN inducer (Cycloferon). In present investigation in the traditional treatment has been included the new preparation - *Polyoxidonium* (60 mg/course) - immunomodulator of number cytokines production and interferons. Before and after treatment we appreciated IFN-status indices, special laboratory tests, clinical efficacy. Monitoring of IFN-status indices, joint syndrome, ultrasonic study indicated theirs considerable improvement after treatment in comparison with initial data and control. In 70% of patients we observed stimulation and normalization of IFN-α and IFN-γ production. Our results suggest that new drug preparation *Polyoxidonium* can be used in rheumatoid arthritis treatment.

30013

INCIDENCE OF AUTOANTIBODIES AGAINST TYPE I IFN IN SLE-PATIENTS AND EPITOPE-MAPPING OF AUTOANTIBODIES AGAINST IFN-α2

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Patients with the autoimmune disease *systemic lupus erythematosus (SLE)* produce antibodies towards various "self" proteins, among others also against cytokines. Our study was focused on the determination of frequencies of autoantibodies recognising the human type I IFNs (alpha 1, alpha 2, beta, omega). Sera from 100 SLE-patients were screened for presence of IFN-binding autoantibodies by an ELISA, using the respective recombinant cytokines as an antigen. The most abundant, with the frequencies of approximately 13%, were antibodies against the subtype IFN-α2 and type IFN-ω. Only seldom, with about 3% incidence, could be detected autoantibodies binding the subtype IFN-α1 and type IFN-β. Antisera containing autoantibodies against IFN-α2 were selected and epitopes of these antibodies were identified. For localisation of epitopes recognised by autoantibodies on IFN-α2 competitive RIA was employed. For this study panel of mapped mouse mAbs to IFN-α2 was used. These mAbs were specific for seven different sites located at both N- and C-termini of the IFN-α2 molecule. In mapping experiments, the extent of competition between autoantibodies and single mAbs for binding of radiolabeled IFN-α2 was measured. In contrast to the therapy induced antibodies against recombinant IFNα2 which preferentially recognise epitopes on the aminoterminal segment of the molecule, autoantibodies were directed against epitopes distributed on the whole immune surface of IFN-α2 protein. This indicates differences between mechanisms responsible for formation of autoantibodies and therapy induced antibodies against IFN-α2.

30010

CYTOKINE-REGULATION OF FAS EXPRESSION IN PANCREATIC ß-CELLS

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Fas-mediated cell death may play an important role in the pathogenesis of autoimmune diseases such as type 1 diabetes mellitus. Fas is expressed in pancreatic ß-cells from patients with recent-onset type 1 diabetes, or in cytokine-exposed rodent and human islets, and renders the ß-cells susceptible to Fas ligand-induced apoptosis. It has been proposed that cytokine-induced Fas expression is mediated by nitric oxide (NO) production. The aim of the present study was to investigate Fas regulation by cytokines in human, mouse and rat ß-cells. For this purpose, we initially analysed Fas mRNA expression by RT-PCR in isolated human islets and FACS-purified rat ß-cells exposed to cytokines with or without N^G-monomethyl-L-arginine (L-MA), an inhibitor of inducible nitric oxide synthase (iNOS). Fas mRNA expression was also determined in islets isolated from wild-type and iNOS knockout mice. In the second part of the study, regulation of Fas promoter activity was determined in FACS-purified rat ß-cells and insulin-producing RINm5F cells. In human islets, Fas mRNA expression was induced by IFN-γ, a cytokine which did not induce iNOS expression. A combination of IFN-γ and IL-1ß induced both iNOS and Fas expression, but Fas induction was not prevented by L-MA. In purified rat ß-cells, Fas expression was induced by IL-1ß alone and was not prevented by L-MA. Islets isolated from wild-type or $iNOS^{-/-}$ mice have similar Fas mRNA content after exposure to a mixture of three cytokines (IL-1ß + IFN-γ + TNF-α). IL-1ß-induction of promoter activity (>10-fold induction; $P<0.01$; n=4-5) is mediated by a region between nucleotides -223 and -54, as suggested by transfection experiments of rat Fas promoter-luciferase reporter constructs into purified rat ß-cells and RINm5F cells. Inactivation of a NF-κB site and a C/EBP site in this region abolished IL-1ß-induced Fas promoter activity in RINm5F cells. Binding of NF-κB and C/EBP to their respective site was confirmed by gel shift assays. These results indicate that cytokine-induction of Fas expression in ß-cells is NO-independent. In rodent ß-cells, IL-1ß is the main inducer of Fas mRNA expression. This effect is mediated, at least in part, by the transcription factor NF-κB.

30015

A CENTRAL ROLE OF MYCOBACTERIAL ADJUVANT-INDUCED MYELOPOIESIS IN EXPERIMENTAL AUTOIMMUNE DISEASES

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Experimental autoimmune diseases often rely on immunisation with the organ-specific auto-antigens in complete Freund's adjuvant (CFA). The killed mycobacteria that are present in this adjuvant are necessary for rapid progression and high incidence of the diseases. In several of these models, including collagen-induced arthritis (CIA), endogenous IFN-γ acts as a disease-limiting factor in the pathogenesis of the disease. The protective effect represents a paradox as it conflicts with the predominantly pro-inflammatory properties of IFN-γ. However, we recently described that the mycobacterial component of CFA elicits extramedullarly myelopoiesis and that this is a pathway by which endogenous IFN-γ exerts a strong protective effect which supersedes its natural disease-promoting effect. We here present evidence that myelopoiesis, elicited by CFA, leads to an enormous expansion of a $Mac\text{-}1^+$ cell population in spleen and liver of IFN-γ receptor-deficient (IFN-γR KO) and wild-type mice, just before the onset of the disease. Later on, $Mac\text{-}1^+$ cells can be harvested from arthritic joints. In fact, inflamed joints almost exclusively contain $Mac\text{-}1^+$ cells, mainly immature and mature monocytes and neutrophils. Isolation of $Mac\text{-}1^+$ cells and stimulation with mycobacterial components revealed high production of TNF-α, a pro-inflammatory cytokine in CIA as well as in rheumatoid arthritis. Remarkably, the production of TNF-α was much more pronounced in the absence of the IFN-γ-receptor even at a single cell basis, as concluded from intracellular flow cytometric analysis. Treatment with anti-TNF antibody inhibited CIA in IFN-γR KO mice. The results demonstrate the importance of myelopoiesis, elicited by killed mycobacteria, in the pathogenesis of CIA and possibly in other autoimmune models that rely on the use of CFA for induction of the diseases.

Toll and apoptosis

31008

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Abstract not available at time of printing.

31004

TROPHIC FACTOR WITHDRAWAL INDUCES A NOVEL PATHWAY: p38 MAPK ACTIVATES NHE1 RESULTING IN INTRACELLULAR ALKALINIZATION, AN EARLY STEP IN APOPTOSIS

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The requirement for cytokines in hematopoiesis is partly attributable to a trophic activity, the protection of cells from apoptosis. Using cytokine-dependent cell lines, we have identified novel events that define early death-promoting activities. Loss of IL-7 or IL-3 induced the phosphorylation and activation of p38 MAP kinase (MAPK). In turn, activated p38 MAPK mediated the phosphorylation of the pH regulator, the Na+/H+ exchanger 1 (NHE1), causing a striking intracellular alkalinization in which cytosolic pH rose steadily, peaking over pH 7.8 before returning to neutrality. A critical outcome of alkalinization was the mitochondrial translocation of the pro-apoptotic protein, Bax. The pH rise triggered a conformational change in Bax, altering charged amino acids in the termini to expose hydrophobic domains. Furthermore, alkalinization produced a transient hyperpolarization of the mitochondrial inner membrane, an effect blocked by oligomycin, inhibitor of the F0F1-ATPase. Functional perturbations included loss of ATP and the accumulation of NADH. Using cells from $Bax^{-/-}$ mice or overexpressing Bcl-2, we showed that cytosolic alkalinization and mitochondrial hyperpolarization are early events independent of the Bcl-2 family. Hence, p38 MAPK/NHE1 is a previously unrecognized death-inducing pathway that upon cytokine withdrawal activates Bax and causes a transient hyperpolarization of the mitochondrial inner membrane, producing severe metabolic stresses potentiating cell death.

31003

CHARACTERISATION OF THE INTERACTION OF THE VACCINIA VIRUS PROTEINS A46R AND A52R WITH MEDIATORS OF IL-1/TOLL SIGNALING.

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The genome of Vaccinia Virus contains a variety of open reading frames encoding potential immunomodulatory proteins. We have previously identified two open reading frames, A46R and A52R, which show sequence identity to the TIR (Toll/Interleukin-1 receptor) domain (Bowie et al., (2000) Proc. Natl. Acad. Sci. USA, in press). Expression of A46R and A52R in mammalian cells specifically inhibited the activation of NFκB in response to IL-1 treatment, while A52R also inhibited NFκB activation by IL-18 treatment and TLR4 expression. In this study we have examined the interaction of these two viral proteins with previously characterised mediators of IL-1/Toll signaling. GST fusion proteins of A46R and A52R were expressed in bacteria and purified using glutathione agarose. The purified fusions were incubated with lysates from 293T cells expressing a variety of IL1/Toll signaling intermediates including MyD88, IRAK, IRAK2, TRAF family members and the kinases NIK and TAK1. Both A46R and A52R formed a complex with IRAK2. The two viral proteins interacted better with a mutated version of IRAK2 lacking the death domain, suggesting this domain exerts a negative regulatory effect on the interaction. In contrast to IRAK2, neither viral protein exhibited significant interaction with IRAK. Ectopic expression of both IRAK and IRAK2 in 293 cells can induce the expression of an NFκB-dependent reporter gene. Expression of A52R specifically inhibited the activation of NFκB by IRAK2 but not IRAK. These results suggest that IRAK2 may be an important target in mediating the inhibitory effects of these two viral proteins on IL1/Toll signalling.

31001

AN ANTAGONIST TOLL-LIKE RECEPTOR 4 (TLR4) DIFFERENTIALLY BLOCKS *M. TUBERCULOSIS*-INDUCED MACROPHAGE RESPONSES

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Mammalian Toll-like Receptor (TLR) proteins mediate the activation of macrophages by a variety of chemically-diverse bacterial products. We previously showed that purified bacterial products, such as Gram-negative lipopolysaccharide (LPS) and mycobacterial lipoarabinomannan (LAM), activate cells via TLR4 and TLR2 (respectively). We now find that viable *M. tuberculosis* (Mtb) bacilli contain distinct ligands that activate cells via both TLR2 and TLR4. In contrast, *M. avium* activate cells only via TLR2. The importance of TLR4-mediated host responses was demonstrated by the finding that TLR4-deficient C3H/HeJ mice are more susceptible to lethal mycobacterial infection compared with normal C3H/OuJ mice. We also observed that the lipid A-like antagonist E5531 inhibited TLR4-dependent Mtb-induced cellular responses. E5531 inhibited LPS- and Mtb-induced NF-κB activation in both murine macrophages and fibroblasts that over-express TLR4, but not TLR2. Interestingly, E5531 blocked LPS- and Mtb-induced TNF-α production in RAW264.7 murine macrophages and primary human alveolar macrophages (AMΦ). In contrast, E5531 did not inhibit Mtb-induced nitric oxide (NO) production in RAW264.7 cells and AMΦ. Mtb could also induce NO production in TLR2-deficient Chinese hamster macrophages, demonstrating that TLR2 did not mediate this response. Lastly, we found that E5531 was capable of inhibiting Mtb-induced macrophage apoptosis in AMΦ. This effect was secondary to the inhibition of TNF-α production by E5531. Together, these data demonstrate that the LPS antagonist E5531 can inhibit TLR4-dependent Mtb-induced cellular activation. Furthermore, our data suggest that Mtb-induced TNF-α production is at least partially dependent on TLR4 signaling, whereas Mtb-induced NO production is independent of TLR2 and TLR4 signaling.

31005

The retinoblastoma protein is an essential mediator that links the human HIN 200 and mouse homologue Ifi 200 genes to cell-cycle regulation.

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The cell growth regulatory activity of interferons (IFN) is the result of the concerted action of a large number of inducible proteins. Among these are those encoded by the human HIN 200 and its mouse counterpart Ifi 200 gene family, which include the human Ifi16 and mouse Ifi204 gene. They encode an 80 kDa and 78 kDa phosphoprotein respectively, that upon IFN-treatment translocate into the nucleus. The Ifi16 protein, like p204, contains two IXCXE motifs that are potential sites for binding to the Rb product and functions as a growth suppressor in sensitive cell lines, as determined by the cell focus assay. Transient overexpression inhibits growth, delays G0/G1 progression into S phase, and impairs E2F-mediated transcriptional activity. Coprecipitation experiments and GST-pull down assay revealed an association in vivo of p204 with the retinoblastoma protein (pRb). Transient p204 overexpression in Rb+/+ mouse embryo fibroblasts (MEF) inhibits cell proliferation, but does not affect cell growth in MEF derived from $Rb^{-/-}$ mice. Two human cell lines, Saos2 and C33A, bearing an inactive pRb, but not primary human embryo fibroblasts, are resistant to the p204 antiproliferative activity. p204 contains two 200 amino acid motifs, designated as type *a* or *b* domains, each containing a canonical Rb binding motif (LXCXE). When dominant-negative mutants at the Rb binding motif were transfected in Rb+/+ MEF, p204 lost its ability to inhibit cell growth, delay cell transition from G1 to S phase, and impair DNA synthesis. Moreover p204 overexpression in Rb+/+ MEF led to a significant decrease of both DHFR and PCNA proteins, two S phase markers. By contrast, this effect was not observed when Rb+/+ MEF were transfected with p204 mutated at both Rb binding sites. These results suggest that the primary target of the IFN-inducible Ifi16 and p204 leading to efficient G1 arrest as well as to blockade of DNA replication from G1 phase is the pRb regulatory system.

31007

Expression of The Large Form of Human 2',5'-Oligoadenylate Synthetase does not confer Antiviral Activity but confers Sensitivity to pIC-induced Apoptosis.

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The 2'-5' oligoadenylate synthetases (OAS) represent a family of interferon-induced enzymes implicated in the mechanism of the antiviral action of interferon. When activated by double-stranded (ds) RNA, these proteins polymerize ATP into 2'-5' linked oligomers with the general formula pppA(2'p5'A)n, $n \geq 1$. Three forms of human OAS have been described corresponding to proteins of 40/46, 69/71 and 100 kDa(p100). The recent cloning of the p100 form have revealed that this form synthesizes mainly the dimer form of 2-5A which is unable to activate the classical effector protein of the 2-5A system. In order to define precisely the function of p100, we have obtained several mouse (3T3 cells) and human cell (HT1080 cells) clones overexpressing constitutively human p100 at levels comparable to IFN-induced cells. Infection of 3T3 clones with EMCV or VSV demonstrated that p100 does not confer any antiviral activity which is obvious in the case of overexpression of p40 and p69 isoforms. This finding illustrates in vivo the absence of a functional relationship between p100 and the activation of RNase L. In contrast, pIC treatment of HT1080 cells expressing p100 shows that expression of this enzyme confers sensitivity to pIC-induced apoptosis. This effect takes place trough caspase 9 activation and subsequent caspase 3 and PARP cleavage. Currently, the involment of RNase L in this effect is under investigation by using RNase L -/- MEFs infected with retroviral constructs expressing p100.

31002

DISTINCT ROLES OF THE IL-2/15Rβ CHAIN AND COMMON γ (γc) CHAINS IN ANTI-APOPTOTIC SIGNALING IN T CELLS

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The cytokines interleukin (IL)-2 and IL-15 play crucial roles in regulating survival, proliferation and apoptosis of T cells during an immune response. During the initial phase of T cell activation, IL-2 is a vital growth/survival factor. However, when there is no further antigen stimulation due to successful immune clearance, IL-2 levels in the microenvironment fall, and T cells undergo apoptosis induced by cytokine withdrawal. Similarly, IL-15 provides critical survival signals for maintenance of memory T cells in the periphery. The receptors for IL-2 and IL-5 are composed of identical signaling subunits, IL-2/15Rβ and γc, and a genetic deficiency in γc results in a severe combined immunodeficiency syndrome. We are interested in how molecular signals delivered by the IL-2/15 receptor complex serve to inhibit apoptosis. Using a chimeric receptor signaling system previously established in IL-2-dependent T cells, we have discovered that neither tyrosine residues nor sequences encoded in the distal tail of IL-2/15Rβ are required for anti-apoptotic signaling, although signals from its proximal tail (i.e., JAK1) are necessary. This result is striking, as considerable prior work has implicated cytoplasmic tyrosine residues of IL-2/15Rβ in growth and survival signaling in other cell types, mediated by the JAK-STAT, MAPK and PI-3 kinase pathways. Therefore, we also examined the role of γc in anti-apoptotic signaling. Although γc is required for IL-2/15-dependent signaling by virtue of its association with JAK3, no other clear functions for its distal tail or tyrosine residues have been described. Here we present evidence that anti-apoptotic signals require specific cytoplasmic domains within γc. These signals are specific to the IL-2/15R complex, because the IL-4 and IL-7 receptors (which also use γc) fail to inhibit apoptosis in this cell line. These data provide the first evidence that γc mediates specific signaling apart from JAK3 activation, and also indicate that γc plays distinct functional roles within different receptor complexes.

31009

TLR4-DEPENDENT, BUT MYD88-INDEPENDENT IL-18 SECRETION FROM KUPFFER CELLS UPON STIMULATION WITH LPS

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IL-18, a potent pleiotropic cytokine, is shown to be secreted by Kupffer cells after cleavage of its biologically inactive precursor upon LPS stimulation in a caspase-1-dependent manner. Here, we investigated the mechanism underlying this LPS-induced IL-18 secretion. Kupffer cells constantly stored IL-18 and constitutively expressed caspase-1 mRNA. Actinomycin D, a transcriptional inhibitor, or cycloheximide, a translation inhibitor, did not inhibit IL-18 secretion from Kupffer cells upon LPS stimulation. Kupffer cells from mice deficient in Toll like receptor (TLR) 4, a signaling receptor for LPS, did not secrete IL-18, while those from mice deficient in MyD88, an adaptor molecule for an LPS signaling pathway, could secrete biologically active IL-18 in a caspase-1-dependent fashion. These results indicate TLR-4-dependent but MyD88-independent IL-18 processing in LPS-activated Kupffer cells.

31006

TOLL LIKE RECEPTOR FAMILY EXPRESSION PATTERN.

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Toll-like receptors (TLR) are a growing family of molecules involved in innate immunity. TLR are structurally characterized by a cytoplasmic Toll/Interleukin-1R (TIR) domain and by extracellular leucine rich repeats. TLR characterized so far activate the MyD88/IRAK signaling cascade. Genetic, gene transfer, and dominant negative approaches have involved TLR family members (TLR2 and TLR4) in LPS recognition and signaling. Accumulating evidence suggests that some TLR molecules are also involved in signaling receptor complexes which recognize components of gram positive bacteria and mycobacteria. However the definitive role of other TLR is still lacking. We have used a systematic approach to determine whether different human leukocyte populations selectively or specifically expressed TLR mRNA. We have analyzed fresh human monocytes, granulocytes, lymphocytes (T and B), NK cells and dendritic cells. Based on expression pattern, TLR can be classified as ubiquitous (TLR1), restricted (TLR2, TLR4 and TLR5) and specific (TLR3). We finally focused our analysis on the the expression and regulation of TLR4 on the cell surface of fresh human monocytes.

Interferons and cytokines in infectious disease II

32004

Mycobacterium tuberculosis infection modulates the type I IFN system

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Cell mediated immunity is essential for the elimination of *Mycobacterium tuberculosis* (*M. tb.*), but the initial course of infection is subject to innate immunity. Type I interferon (IFN) is a key cytokine for the innate immune response, but its role at the outset of *M. tb.* infection is poorly understood. *Ex vivo* infections of peripheral blood monocytes, monocyte-derived macrophages, and alveolar macrophages caused activation of ISGF3. Activation of ISGF3 also occurred upon infection of the human monocytic THP-1 cell line, which we used as a model for the effect of infection on the type I IFN system. Furthermore, infected undifferentiated THP-1 cells and blood monocytes exhibited greater activation of ISGF3 than uninfected cells in response to subsequent treatment with IFNα. These results strongly suggested secretion of and response to type I IFN upon infection. Several experiments, including measurement of IFNα-regulated gene expression, confirmed this autocrine pathway. However, infection alone did not lead to formation of STAT-1 homodimers, and actually reduced formation of STAT-1 homodimers in response to a subsequent pharmacological dose of IFNα. We also found that *M. tb.* infection increased the abundance of total STAT1, STAT-2 and IRF-9 proteins, and led to tyrosine phosphorylation of STAT-1 and STAT-2, in monocytes and macrophages. However, compared to uninfected cells treated with IFNα, *M. tb.* infection led to decreased tyrosine phosphorylation of STAT-1 upon further treatment with IFNα, both in monocytes and macrophages, while the levels of tyrosine phosphorylated STAT-2 are reduced in macrophages but increased in monocytes. The overall levels of ISGF-3 and STAT-1 DNA-binding activities thus were accounted for by the combined effects of changes in subunit abundance and tyrosine phosphorylation. These effects were not caused by infection with avirulent *M. bovis* BCG. Further examination of induced type I IFN secretion, preferential ISGF-3 activation, and their consequences will help understand whether these effects of *M. tb.* infection on type I IFN signal transduction are part of a defensive or pathogenic host response.

32001

TGF-β MEDIATES THE MYCOBACTERIA CELL ENTRY PROTEIN (MCEp) INHIBITION OF THE IMMUNE CELLULAR RESPONSE, NITRIC OXIDE PRODUCTION AND INCREASE OF HIV REPLICATION.

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MCEp mycobacteria cell entry protein was originally described as a protein capable of mediating the *M. tuberculosis* (Mtb) entry and survival within phagocytic and non phagocytic cells. It is one of few M.tb proteins known to be related with its virulence, but the mechanism underlying these effects remain obscure. Attempting to understand the role of MCEp in the immune response of tuberculosis patients and PPD-, PPD+ individuals we tested the proliferative response and NO production by PBMC to MCEp in vitro. On the other hand, as it is well known that M.tb infection has an important influence on HIV replication in macrophages, we have studied the effects of MCEp addition to human monocyte/macrophage cultures infected with BAL-HIV. Our results show that MCEp was not recognized by PBMCs from tuberculous or *M. tuberculosis*-infected individuals. MCEp did not induce IL-2, IFN-γ TNF-α or IL-10. However PBMCs-stimulated by MCEp produced significantly more TGF-β than unstimulated cells. In addition, MCEp was able to inhibits the capacity of Con A to induce PBMCs' proliferation This inhibition was restored by neutralizing TGF-β. Human macrophages-stimulated by MCEp produced significantly more TGF-β than unstimulated cells (4860pg/ml X 1782pg/ml). TGF-β expression was also detected in the cytoplasm of human macrophages by immunohistochemistry technique. MCEp also inhibited the NO-induced by IFN-γ plus TNF-α and Mtb antigen in J774 cells (27.6 ng/ml x 0.6 ng/ml). HIV (Bal strain) replication inside human macrophages was significantly augmented by MCEp. The percentage of HIV-replication in MCEp-stimulated cells compared to unstimulated HIV-infected cells were respectively –36.7%, 225.8% and 26.5% respectively at 7, 14 and 21 days after macrophage infection *in vitro*. Both effects of MCEp can be restored by neutralizing TGF-β. In conclusion a single *M. tuberculosis* protein, MCEp, blocks the host immune response against M.tb and augment HIV replication though TGF-β production.

32006

SUPPRESSED PRODUCTION OF LEPTIN IN TB-PATIENTS CORRELATES WITH T-CELL UNRESPONSIVENESS

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Cachexia, a characteristic feature of tuberculosis (TB), is known to suppress cellular immunity. The exact mechanisms are unknown, but leptin, a cytokine produced by adipocytes, may be involved Nutritional status, cytokine production and plasma leptin were evaluated in 59 TB patients (30 males) and 20 health matched controls in Jakarta, Indonesia The median body mass index was 17 0 in TB patients compared with 19.2 in controls, the estimated fat percentages were 10.5 and 21 4 ($p < 0.001$) Plasma concentrations of leptin were variable (range 75 - 6035 pg/ml) and were correlated with fat mass ($r = .78$; $p < .001$). Even when corrected for fat mass, leptin was significantly lower in untreated patients than in controls (106 vs 364 pg/ml/kg fat; $p = .015$) Interestingly, in individual patients the ratio of plasma leptin to fat mass increased during two months of treatment ($p < .001$). Plasma leptin was correlated with the size of the tuberculin-skin-test ($r = .455$; $p = 0.044$) In addition, changes in plasma leptin were parallel to the rise in ex-vivo production of IFNγ. No correlation could be found between leptin and subjective anorexia, or between leptin and the ex-vivo production of monocyte-derived cytokines TNFα, IL-1β, IL-1Ra and IL-6. Together, these results indicate that patients with active TB have a reduced production of leptin, which reverses after start of treatment Decreased concentrations of leptin seem to mediate T-cell unresponsiveness, which may be one of the causes for a worse outcome of TB in patients with a poor nutritional status

32007

CD40-CD40L INTERACTIONS ARE REQUIRED FOR HOST DEFENSE AGAINST DISSEMINATED CANDIDA ALBICANS INFECTION. THE ROLE OF NITRIC OXIDE

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CD40/CD40L interactions stimulate synthesis of proinflammatory cytokines such as TNF, interleukin-1 (IL-1) and IL-6, which have been shown to exert protective effects during infection with *Candida albicans* We investigated the role of CD40-CD40L interactions during disseminated candidiasis in CD40L knock-out (CD40L-/-) mice When wild-type and knock-out mice were infected i.v. with $5x10^5$ CFU of *C. albicans*, the CD40L-/- mice had a significanlty higher mortality compared with control CD40L+/+ mice, accompanied by a significantly increased yeast load in the kidneys of CD40L-/- mice on day 15 of infection (7.2 +/- 0.7 vs. 5.7 +/- 0 6 log CFU/g, $p<0.05$) The peak TNF plasma concentrations were measured on day 3 of infection, when plasma TNF levels were significantly lower in the CD40L-/- mice compared with control CD40L+/+ (40 +/- 12 vs. 92 +/- 23 pg/ml, $p<0.05$). The role of nitric oxide (NO) in mediating the effects of CD40 was investigated by blocking CD40 during experimental candidiasis in mice deficient in NO-synthase (iNOS-/- mice). Whereas an antagonistic anti-CD40 antibody significantly increased candidal growth in the kidneys of iNOS+/+ mice, no effects could be observed in iNOS-/- mice, arguing for an important role of NO in mediating CD40 effects. Indeed, the production of NO by macrophages of CD40L-/- mice after stimulation with a combination of *C. albicans* and IFNγ was significantly lower than that of CD40L+/+ mice (5.9 +/- 2.3 vs 10 2 +/- 3.1 μM/ml, $p<0.05$). Intracellular killing of *C. albicans* during 4h of incubation with macrophages was significantly impaired in CD40L-/- mice compared with control animals (53% reduction, $p<0.05$), whereas addition of sodium nitroprusside, an NO donor, to the macrophages of CD40L-/- mice completely restored the normal candidicidal activity. In conclusion, lack of CD40-CD40L interactions results in increased susceptibility to disseminated infection with *C. albicans*, through inadequate production of protective proinflammatory cytokines and macrophage-derived NO.

32003

THE INTERLEUKIN-18/INTERFERONγ PATHWAY IS ESSENTIAL FOR THE DEFENSE AGAINST DISSEMINATED CANDIDIASIS

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Disseminated infection with *Candida albicans* is recognized with increased frequency especially in immunocompromised hosts. Interferon-γ (IFNγ) is a key cytokine for innate and acquired resistance to candidiasis, and the newly-described cytokine interleukin-18 (IL-18) is an essential stimulus of IFNγ production. The role of endogenous IL-18 in the cascade leading to IFNγ production and in the defense against disseminated candidiasis, has been investigated. When human whole blood was stimulated with heat-killed *C. albicans*, neutralization of endogenous IL-18 by its natural inhibitor IL-18 binding protein (IL-18bp) strongly reduced IFNγ synthesis in a dose-dependent manner, up to a maximal inhibition of 60-70% ($p<0.03$). Also, neutralization of endogenous IL-12 by neutralizing monoclonal antibodies reduced *Candida*-induced IFNγ production. Simultaneous treatment of cells with IL-18bp and anti-IL-12 antibodies reduced the IFNγ production by more than 90% ($p<0.01$). During systemic infection of mice with $5x10^5$ CFU *C. albicans*/mouse, a peak of 277 ± 110 ng/ml IL-18 could be measured 8h after the challenge, which was not present in TNF-/-LT-/- mice. Neutralization of endogenous IL-18 by polyclonal antibodies resulted in an increased yeast load in the kidneys of anti-IL-18-treated mice compared to controls on day 7 of infection (4.9 +/- 0.2 vs. 4.0 +/- 0.3 CFU/g, $p<0.05$). The important role of IFNγ in mediating these effects of endogenous IL-18 was demonstrated by the lack of effect of the anti-IL-18 antibody treatment in IFNγ-deficient mice. These data provide new insight in the cytokine cascade induced by *C. albicans*, and demonstrate an important IL-18-dependent role of IFNγ in the immune defense against disseminated candidiasis.

32002

IMPROVED SURVIVAL AND IMMUNITY IN INTERLEUKIN-6 (IL-6) DEFICIENT MICE SUBJECTED TO BURN TRAUMA.

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We recently reported that a higher percentage of female, than male, mice subjected to a moderate size 15-20% total body surface area dorsal scald injury fail to survive. The female mice had suppressed cell mediated immune responses (delayed type hypersensitivity (DTH) and mitogen-mediated splenocyte proliferation) and high circulating levels of IL-6. Since high levels of IL-6 suppress T lymphocyte function and correlates positively with increased mortality in burn patients, we chose compare survival and immunity in burn injured mice that are deficient for the IL-6 gene ($IL\text{-}6^{-/-}$) vs. wild type ($IL\text{-}6^{+/+}$). Nearly all (96%) of the burned $IL\text{-}6^{+/+}$ mice died during the 10 day time period examined, most within the first 4 days. In contrast, only 65% of the $IL\text{-}6^{-/-}$ died ($p<0.05$ vs. $IL\text{-}6^{+/+}$ mice). All of the sham injured mice survived, regardless of IL-6 deficiency. While the cause of death has yet to be determined, the improved survival in $IL\text{-}6^{-/-}$ mice, relative to $IL\text{-}6^{+/+}$, was comparable what we saw after anti-IL-6 antibody treatment. In addition, we found that the DTH response was greater in burn injured $IL\text{-}6^{-/-}$ mice than in burned $IL\text{-}6^{+/+}$ mice. The DTH response in $IL\text{-}6^{+/+}$ mice was <5% of the sham-injured $IL\text{-}6^{+/+}$ mice ($p<0.01$), a levels similar to the response in naive mice. In contrast, the DTH response in burn-injured $IL\text{-}6^{-/-}$ mice was 43% of the response generated in sham-injured $IL\text{-}6^{-/-}$ mice and 8-fold greater than in burn-injured $IL\text{-}6^{+/+}$ mice ($p<0.01$). These observations were also similar to what wild type mice given anti-IL-6 antibody after burn injury. The results shown herein confirm that IL-6 is a critical regulatory of cell mediated immunity and survival after burn trauma. Future studies are likely to show the therapeutic efficacy of blocking IL-6 in critically burned patients.
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32005

USE OF RECOMBINANT HUMAN IL-1β IN THE TREATMENT OF PATIENTS WITH POSTTRAUMATIC INFECTIOUS COMPLICATIONS

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Aim: low doses of pro-inflammatory cytokine IL-1β are capable of enforcing host resistance and protect against death from either gram-negative or gram-positive bacteria, Candida albicans. Patients with sepsis in stage of "immune paralysis"; and infectious complications after severe trauma had reduced endogenous production of IL-1 and other pro-inflammatory cytokines. For this reason we studied efficacy and safety of the rh IL-1β in treatment of 30 patients with different posttraumatic infectious complications (18 patients) and subacute sepsis (12 patients).
Methods: the course of treatment with rh IL-1β consisted of 3 IV infusions TTW, 5 ng/kg per one day infusion.
Results: immunological tests after the course of rh IL-1β showed the increasing CD3 lymphocyte counts (in 73,3% patients), activation of ex vivo peripheral blood lymphocytes proliferation (76,7%), increase in the endogenous production of IL-2 (70%), stimulation of chemotaxis and phagocytosis of peripheral blood neutrophils (80%). Clinical improvement after rh IL-1β therapy in patients with posttraumatic immunodeficiency was accompanied by quick regression of the systemic inflammatory syndrome (86,7%), increase in wound healing (93,3%), correction of the anemia (63,3%), stabilization of psychosomatical status (80%), reduction of the duration of antibiotic treatment (83,3%).
Conclusion: this investigation shows the new approach to the treatment of posttraumatic infectious complications using cytokine therapy with recombinant human IL-1 beta.

Viral anticytokine strategies

33009

ANTI-CYTOKINE STRATEGIES BY VIRUSES

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33006

DNA Microarrays and Hepatitis C Virus Infection: Insights Into Mechanisms Of Pathogenesis And Interferon Resistance

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Hepatitis C Virus (HCV) infects greater than 2% of the world population. The only approved therapy for HCV infection is interferon (IFN), often now together with ribavirin. Unfortunately the majority of infected individuals fail to respond to these therapeutic regimens. To define molecular mechanisms by which HCV evades IFN treatment, we examined the PKR protein kinase regulatory pathway. PKR is an IFN-induced gene, known to be an important arm of the IFN antiviral response. We found that the NS5A protein of HCV genotypes 1a and 1b can bind to and inactivate PKR. Importantly, NS5A can confer, in trans, IFN resistance to an otherwise IFN sensitive virus. Taken together, these data support the hypothesis that NS5A function is critical to viral survival and IFN resistance. More recently we have uncovered another crucial biological role for NS5A. NS5A contains proline rich (PxxP) domains that mediate the interaction between NS5A and the SH2/SH3 domain containing Grb2 adaptor protein in response to epidermal growth factor (EGF), suggesting that the HCV NS5A may be a novel interceptor of cellular signaling. Using DNA chips with 15,000 unique human genes, we have expanded these studies to gain further insights into the role of NS5A on HCV pathogenesis and drug susceptibility. We observed that NS5A repressed the induction of selected IFN induced genes. Moreover, expression of NS5A alone caused the differential regulation of a number of cellular genes. Genes differentially regulated include those involved with transcriptional, translational, extracellular matrix, and cellular signaling networks. Finally, in collaboration with Dr. Nelson Fausto, we have initiated microarray studies on interferon-treated primary fetal hepatocyte cells that are productively infected with both HCV and an infectious HCV molecular clone.

33003

DISRUPTION OF HOST DOUBLE-STRANDED RNA SIGNALING BY HEPATITIS C VIRUS

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Hepatitis C virus (HCV) is a global public health threat with 2% of the world population persistently infected with the virus. The mechanisms of HCV persistence are unclear, but recent studies suggest that persistence is linked to an ability of the virus to overcome host antiviral pathways induced by double-stranded RNA (dsRNA) and interferon (IFN). We examined the response to dsRNA and IFN in cell lines engineered to express the HCV genome or subgenomic fragments of HCV. Gel-shift analyses revealed that HCV genome expression blocked the dsRNA and virus-induced activation of interferon regulatory factor-1 (IRF-1) and a dsRNA-activated factor (DRAF), and prevented the binding of these factors to the IFN-stimulated response element of an IFN-responsive promoter. HCV protein expression resulted in alteration of IRF-1 localization from a general nuclear pattern to redistribution around the nuclear membrane, but did not affect overall IRF-1 expression. Thus, HCV may prevent IRF-1 activation through a process that involves re-directing IRF-1 localization within virus-infected cells. Our results suggest that HCV may disrupt host cell dsRNA signaling pathways during persistent infection. The nature of the DRAF, and the role that individual HCV protein products play in IRF-1 and DRAF regulation will be presented.

33001

FUNCTIONAL CHARACTERIZATION OF A NOVEL SECRETED CHEMOKINE BINDING PROTEIN ENCODED BY A HERPESVIRUS

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Molecular mimicry of cytokines and cytokine receptors is a strategy adopted by large DNA viruses to modulate the host immune response. Murine gammaherpesvirus 68 (MHV-68) is related to the human pathogen Kaposi's sarcoma-associated virus, which encodes chemokine homologs to modulate chemokine activity. We show that the MHV-68 M3 gene encodes a secreted protein that binds chemokines and blocks their interaction with cellular receptors and signal transduction. Interestingly, the M3 protein has no sequence similarity to the seven-transmembrane-domain cellular chemokine receptors nor to the poxvirus 35-kDa chemokine binding protein (CKBP) that binds CC chemokines. The herpesvirus CKBP exhibits a unique broad binding specificity, including CC, CXC, C and CX3C chemokines. We have analyzed the effect of a targeted M3 gene disruption on viral pathogenesis in infected mice. An M3 deficiency had surprisingly little effect on lytic cycle replication in the respiratory tract or on the initial spread of the virus to lymphoid tissue. However, the proliferation of latently MHV-68-infected B cells in the spleen did not occur, and depletion of CD8+ T cells resulted in partial restoration of this deficiency. Thus chemokine inhibition by M3 in vivo prevents immune elimination of virus-infected cells during the establishment of latency. M3 is the first CKBP identified in herpesviruses and represents a novel protein structure with the ability to bind all subfamilies of chemokines in solution and has potential therapeutic applications. The MHV-68 chemokine inhibitor represents a novel virus immune evasion mechanism that provides insights into viral pathogenesis and new strategies of immune modulation.

33007

HHV-8 encoded vIRF-1 represses the interferon antiviral response by blocking IRF-3 interactions with the CBP/p300 coactivator

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Human herpes virus 8 (HHV-8) has developed unique mechanisms for altering cellular proliferative and apoptotic control pathways by incorporating viral homologs to several cellular regulatory genes into its genome. One of the important pirated genes encoded by the ORF K9 reading frame is a viral homolog of the interferon regulatory factors (IRF), a family of cellular transcription proteins that regulates expression of genes involved in pathogen response, immune modulation and cell proliferation. vIRF-1 has been shown to downregulate the interferon- and IRF-mediated transcriptional activation of ISG and murine IFNA4 gene promoters. In this study we demonstrate that vIRF-1 efficiently inhibited virus-induced expression of endogenous interferon B, CC chemokine RANTES and CXC chemokine IP-10 genes. Co-expression analysis revealed that vIRF-1 selectively blocked IRF-3 but not IRF-7-mediated transactivation. vIRF-1 was able to bind to both IRF-3 and IRF-7 *in vivo* as detected by coimmunoprecipitation analysis, but did not affect IRF-3 dimerization, nuclear translocation and DNA binding activity. Rather, vIRF-1 interacted with the CBP/p300 coactivators and efficiently inhibited the formation of transcriptionally competent IRF-3-CBP/p300 complexes. These results illustrate that vIRF-1 is able to block the early stages of the IFN response to virus infection by interfering with the activation of immediate early IFN genes.

33008

A NOVEL MECHANISM OF HEPATITIS C VIRUS INTERFERON RESISTANCE: INDUCTION OF EXPRESSION OF THE CXC CHEMOKINE, INTERLEUKIN-8, BY THE NON-STRUCTURAL 5A PROTEIN

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Hepatitis C virus (HCV), a major cause of liver disease worldwide, is frequently resistant to the antiviral interferon (IFN). The HCV non-structural (NS) 5A protein has been implicated in HCV antiviral resistance in many studies. NS5A antagonizes the IFN antiviral response *in vitro*, and one mechanism is via inhibition of a key IFN-induced enzyme, PKR. Mutants of NS5A lacking the amino terminus have also been reported to possess non-specific transcriptional activation functions. In the current study we determined if NS5A uses other strategies to subvert the IFN system. Tetracycline regulated expression of full-length NS5A proteins in human cells had no effect on IFN induction of the ISGF-3 transcription factor, STAT-1 phosphorylation, PKR, and MHC class 1 antigens. However, levels of IL-8, previously shown to inhibit the antiviral actions of IFN in vitro, were elevated in HCV-infected patients as compared to normal healthy subjects, and were also higher in patients who were non-responders to IFN therapy as compared to responsive patients. NS5A expression in human cells induced IL-8 mRNA and protein, and this effect correlated with the anti-IFN function of NS5A in a *trans* rescue assay previously developed in our laboratory. NS5A induced transcription of a reporter gene driven by the IL-8 promoter, and mutant NS5A proteins lacking the amino terminus displayed heightened transactivation. Characterization of the transcription factors involved in this process is in progress. This study provides the first evidence for an alternate mechanism of NS5A-induced antiviral resistance, as well as providing a biological effect for the transcriptional activity of the NS5A protein. In summary, NS5A appears to perturb the IFN system by at least two mechanisms, suggesting that during HCV infection, viral proteins may induce cytokines that contribute to HCV antiviral resistance and pathogenesis.

33005

Expression of Hepatitis C Virus Proteins Inhibits Signal Transduction through the Jak-STAT Pathway

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Hepatitis C virus (HCV) infection is a leading cause of liver disease worldwide. Currently, interferon-alpha (IFN-a) in combination with ribavirin is the only approved therapy for chronic hepatitis C, with a sustained response in 30-40% of patients only. The mechanisms of viral persistence and the pathogenesis of hepatitis C are poorly understood. We established continuous human cell lines allowing the tightly regulated expression of the entire HCV open reading frame under the control of a tetracycline-responsive promoter. Using this in vitro system we analyzed the effect of HCV proteins on IFN induced intracellular signaling. Expression of HCV proteins in these cells strongly inhibited IFN-a induced signal transduction through the Jak-STAT pathway. Inhibition occurred downstream of STAT tyrosine phosphorylation. Inhibition of the Jak-STAT pathway was not restricted to IFN-a induced signaling, but was observed in leukemia inhibitory factor induced signaling through Stat3 as well. By contrast, TNF-alpha induced activation of the transcription factor NF-kB was not affected. Interference of HCV with IFN-a induced signaling through the Jak-STAT pathway could contribute to the resistance to IFN-a therapy observed in the majority of patients and may represent a general escape strategy of HCV contributing to viral persistence and pathogenesis of chronic liver disease.

33004

HCV NS5A NONSTRUCTURAL PROTEIN PERTURBS MULTIPLE SIGNALING PATHWAYS BY TARGETING GRB2 ADAPTOR PROTEIN AND GAB1 SIGNALING COMPLEX

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Hepatitis C virus (HCV) is the leading cause of chronic liver diseases and a major public health hazard worldwide, while the molecular mechanism of HCV pathogenesis remains poorly understood. However, in the majority of chronic patients HCV is resistant to interferon-α treatment, the primary therapy available. Recent studies suggest HCV may block interferon signaling. Our previous study showed that NS5A nonstructural protein of HCV contains two highly conserved proline-rich Src homology 3 (SH3) domain-binding motifs, one of which specifically interacts with Grb2 adaptor protein in a SH3 domain-/ligand-dependent manner. Upon EGF stimulation of mammalian cells, NS5A, but not a proline-rich motif NS5A mutant, transiently associates with Grb2 and prevents sustained activation of Erk1/2 by EGF. NS5A also associates with Gab1 signaling complex upon EGF treatment and may regulate downstream pathways. A model is suggested in which NS5A targets Grb2, a master switch involved in multiple signaling pathways, and thus affecting multiple cellular processes. Finally we found that NS5A inhibits interferon-stimulated, ISRE-dependent gene expression. These findings highlight a potential role of NS5A in HCV pathogenesis and interferon resistance.

33002

INDUCTION OF A NOVEL CELLULAR HOMOLOG TO INTERLEUKIN-10, AK155, BY TRANSFORMATION OF HUMAN T CELLS WITH HERPESVIRUS SAIMIRI

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Herpesvirus saimiri-transformed T lymphocytes retain multiple normal T-cell functions, while only a few changes have yet been described in comparison to non-transformed parental cells. By subtractive hybridization, we have isolated a novel cellular gene, *ak155*, showing 25% identity and 47% similarity to interleukin-10 on amino acid level. The gene maps to a cluster of interleukin-10 related genes on human chromosome 12q15 between the gamma interferon and IL-TIF loci. Specifically herpesvirus saimiri-transformed T cells strongly overexpress *ak155* and secrete the protein into the supernatant. In other T-cell lines and in native peripheral blood cells, but not in B cells, *ak155* is transcribed at low levels. AK155 forms homodimers similarly to interleukin-10. As a lymphokine, AK155 may contribute to the transformed phenotype of human T cells after infection by herpesvirus saimiri. AK155 belongs to a growing novel family of interleukin-10 related cytokines of cellular or viral origin.

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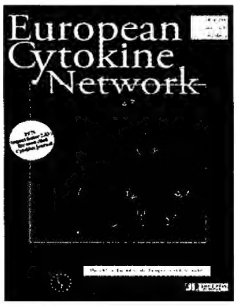

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